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# THE EFFECT OF ATROPIN UPON SKELETAL MUSCLE TONUS

LOYAL DAVIS AND LEWIS J. POLLOCK

*From the Departments of Surgery, Nervous and Mental Diseases and Laboratory of Surgical Research of Northwestern University Medical School*

Received for publication December 28, 1929

It has been noted for many years that atropin, hyoscin, scopolamin and duboisin have a peculiarly specific action upon the rigidity of the muscles in patients suffering from paralysis agitans and Parkinsonism following encephalitis lethargica. In both of these conditions the rigidity is diminished and in many cases completely disappears. The manner in which these drugs affect this hypertonicity has been unknown.

Kuré and Shinosaki (1) believe that the diminution of tone in Parkinsonism as the result of the administration of atropin is due to the paralyzing action of the drug on hypothetic parasympathetic fibers which end in skeletal muscle. There is some evidence of the disturbance of other sympathetic and parasympathetic functions in Parkinsonism. Sialorrhea is a constant symptom of the condition. Increased sweating is present and seborrhoea is so frequently observed that it is often an important early symptom.

Walshe (2) has shown that intramuscular injections of novocaine, so graded as to paralyze the afferent nerve fibers in the muscle and to leave the activity of the motor nerve fibers unimpaired, abolishes or greatly diminishes the rigidity of paralysis agitans. From this, he believes that the integrity of the proprioceptive reflex arc from the muscle is essential to the development and maintenance of Parkinsonian rigidity.

In confirmation of the work of Walshe, we (3) have found that section of the fourth, fifth, sixth, seventh and eighth cervical, first, second, third and fourth thoracic posterior roots abolishes the rigidity in the upper extremity of a patient suffering from Parkinsonism following encephalitis lethargica.

Modifications of tone by the action of drugs can be studied excellently in a decerebrate animal, in which it is possible to destroy separately the muscle proprioceptors, the labyrinths and the neck reflexes.

Hugget and Mellanby (4) were unable to affect the tonus of intact anesthetized cats or of decerebrate animals by the injection of 5 mgm. of atropin. In the course of some former work we too were unable to affect the rigidity of decerebrate cats by the injection of 1 to 2 mgm. of atropin. It was felt that since in Parkinsonian rigidity it had been observed that it was necessary at times to administer hyoscin for a day or two before its full effect was noted in untreated cases, the experiment should be repeated and should be modified by first atropinizing the animal and then performing the decerebration. Accordingly, a cat was given 2 mgm. of atropin hypodermically three times a day for four days and it was then decerebrated by the anemic method (5). During the four days of the administration of atropin, aside from the difficulty of vision and increased thirst, nothing abnormal was noted in the behavior of the cat. Following this experiment nine other animals were subjected to the same procedure and the results were constant in all.

In sharp contrast to the normal decerebrate cat, it was seen that when the an mal was posed upon its back, the hind legs fell into a flexed position and very little rigidity was present. When passively extended and allowed to fall the legs quickly returned to their original position without resistance. The forelegs were rigidly extended. This contrast of action upon fore and hind legs permitted an analysis of the action of atropin. The constant difference of this action upon fore and hind legs could only mean that there was some mechanism responsible for the persistence of the rigidity in the forelegs which was not present in the hind legs.

In a former communication (6) we pointed out that when the labyrinths have been destroyed and an animal decerebrated, when suspended with the head passively extended, marked rigidity was present in all the extremities. When the passively extended head was allowed to drop it fell into a flexed position and the forelegs immediately flexed and remained so as long as the head was allowed to remain in that position. The hind legs, however, became even more strongly extended. It can be seen, therefore, that the labyrinthine reflexes act with far greater force upon the forelegs either through the neck reflexes or directly. Indeed, it may be found that they do not at all influence the tone of the hind legs directly. The neck reflexes, however, have a decided action upon the tone of the hind legs either directly or indirectly. The action of the neck reflexes upon the fore and hind legs does not produce similar patterns of posture. Whereas, anteflexion of the head produces flexion in the forelegs, it is accompanied by greater extension in the hind legs and vice versa. Here then is found a mechanism, labyrinthine reflexes, which may produce extension in the forelegs and when removed permits a lasting position of flexion without greatly changing the tone in the hind legs. The increased tone in the hind legs, therefore, is dependent more upon other reflex activities; that is, neck and other muscle proprioceptive reflexes.

How much the neck reflexes influence the extensor tone of the hind legs may easily be determined by severing the appropriate cervical posterior roots and destroying the labyrinths. The extensor tone in the hind legs may then be compared with that in an animal with the neck reflexes intact. Magnus found that in an otherwise normal animal with the labyrinths removed and the first three cervical posterior roots severed, the forelegs were always flexed in a "kangaroo" position. Curiously, he found that when such an animal was decerebrated, the forelegs had sufficient extensor tone to support the weight of the animal.

This observation did not coincide with our conception of the integration of the tonic reflexes. We felt that if the normal activity of an animal with the labyrinths removed and the neck reflexes destroyed was represented by flexion in the forelegs, decerebration would not change the distribution of tone. It is known that in such animals as normally possess a flexor attitude of the forelegs, such as the sloth or monkey, decerebration is followed by an increase of tone *in flexion*. We therefore destroyed the labyrinths and severed the first four cervical posterior roots in a number of cats. They were then decerebrated. We found that in such animals the forelegs were in a position of flexion but that the hind legs although not as strongly extended as in an ordinary decerebrate animal, had a preponderance of tone in the extensors. This indicated that the forelegs maintained an outstreteched position of increased extensor tone chiefly through the mediation of the neck and labyrinthine reflexes. On the contrary, extensor tone in the hind legs was mediated to a larger degree by the muscle proprioceptors.

The contrast between the action of atropin upon the fore and hind legs may then be due to the fact that it diminishes only that type of tone which is not mediated by the labyrinthine or neck reflexes. It remained to determine whether atropin produces any quantitative or qualitative action upon the labyrinthine or neck reflexes, to determine its action upon the muscle proprioceptive reflexes and upon nociceptive reflexes.

When an extremity is deafferented, the tonic reflex activities are mediated through the neck and labyrinthine reflexes and the crossed reflexes from the opposite side of the body. The comparison of the extensor tone developed by the labyrinthine reflexes acting upon the deafferented limb with the animal upon its back, before and after atropinization, will indicate the effect of the drug upon the labyrinthine reflex. To further isolate the possible action of atropin upon the labyrinth, the neck reflexes may be destroyed by severing the first four cervical posterior roots and the tone in the forelegs present after the atropinization compared with that in an animal to which no atropin has been given. To isolate the action of atropin upon the muscle proprioceptors it was necessary only to study its action upon a decerebrate animal with the labyrinths destroyed and the first four cervical posterior roots severed.

In all of the ten decerebrate cats which were atropinized and in all of the ten other decerebrate cats, some with neck and labyrinthine reflexes destroyed or an extremity deafferented, active knee jerks, pinna, phlyctenular, and withdrawal reflexes were present. In fact, they were more active than in similar animals which had not been atropinized. It was obvious that atropin did not affect the reflex arc of such reflexes either centrally or peripherally.

In ten decerebrate atropinized cats and in four decerebrate atropinized cats with a deafferented extremity and in three animals decerebrated after destruction of the neck reflexes, there was no qualitative change in the labyrinthine reflexes elicited by turning the occiput down with the animal posed upon its back. No quantitative change in the extensor tone developed by a labyrinthine reflex in a deafferented extremity or in animals with the neck reflexes destroyed was found following atropinization.

No qualitative change was found in the neck reflexes after atropinization in any of the animals with the first four cervical posterior roots intact.

When one foreleg was completely deafferented by section of the first twelve posterior roots and when two weeks later the animal was atropinized and decerebrated, the extensor tone in the deafferented forelimb was less than that in a normal animal but the extensor tone developed by turning the occiput down measured 300 grams which was the amount found to be developed in similar animals not atropinized.

After the neck reflexes were destroyed in three cats which were subsequently atropinized and decerebrated, the hind legs were found flexed. When they were passively extended and allowed to drop, they fell promptly. The forelegs were rigidly extended and no difference could be found between animals which had been given atropin and those not atropinized.

However, if the labyrinths were removed in such animals all of the extremities were held in a flexed position with the animal lying upon its back. When the extremities were passively flexed further they remained in the new position. Slight extensor rigidity was present only in the hind legs. When they were passively flexed to an angle of 60 degrees, resistance was met and this remained at the same level throughout further flexion.

Of course in these latter animals no neck or labyrinthine reflexes were present and tone was developed only by muscle proprioceptors. Atropin, therefore, acted alike upon fore and hind legs. A clinical method for the examination of a muscle proprioceptive reflex is found in the Stütz reflex described by Schöen and Pritchard (7). When the foot pad is pressed upon, extensor tone is produced and each succeeding pressure is followed by greater and greater extension of the limb until marked extensor rigidity is developed in the extended limb. This reflex was absent in all extremities in all twenty atropinized animals. It was the only reflex activity which was constantly absent, irrespective of what other reflex activities were present.

It seemed apparent, therefore, that atropin did not act on the central connections between neck, labyrinthine, nociceptive or body reflexes, inasmuch as the former three could be elicited without qualitative or quantitative change and crossed extensor thrusts could be evoked easily. It did not act upon the muscles directly as so many reflex activities were not only present but were increased. Only one reflex activity was always affected, the muscle proprioceptive reflex. When all other tonic reflex activities of the forelegs were destroyed, leaving only the muscle proprioceptive reflexes, then tone was diminished in them just as in the hind legs where the hypertonicity is mediated largely by muscle proprioceptive reflexes. There is therefore no need for postulating the existence of antidromic tonic fibers in the posterior root in this case because the results may be explained easily on the basis of ordinary reflex activity.

## CONCLUSIONS

1. Atropin diminishes decerebrate rigidity by its action upon the muscle proprioceptor arc.

2. Its action in diminishing the rigidity of Parkinsonism is probably the result of the same action.

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# THE FUNCTIONAL ANALYSIS OF A SYMPATHETIC GANGLION OF THE TURTLE BY THE CATHODE RAY OSCILLOGRAPH

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In a previous communication (Heinbecker and Bishop, 1929) the action potentials of the cervical sympathetic trunk of the turtle were described. The compound conducted action potential consists of three main potential components designated $B_1$ (the first or $A$ component present in certain nerves being here absent), $B_2$ and $C$.[2] Of these three the $B_1$ component is only occasionally present in the turtle sympathetic. The properties of the fibers giving rise to this $B_1$ potential have been found to be similar to those of the ordinary myelinated fibers giving rise to the $A$ elevation of the sciatic nerve of the turtle by the author. The type of fiber responsible for the $B_1$ wave was shown to be the larger relatively thinly myelinated one seen in autonomic nerve trunks, ordinarily inferred to be afferent in type. The $B_2$ group was found to arise from the smaller thinly myelinated axons always closely associated with islands of unmyelinated axons considered the source of the $C$ potential wave, these two groups presumably constituting the autonomic efferents of this nerve.

The present paper deals with a study of the conduction of the $B_1$, $B_2$ and $C$ potential elements through the superior cervical ganglion of the turtle[3] in the afferent and efferent directions.

MATERIALS AND METHOD. The turtles studied were the Chrysemys picta, the Chrysemys marginata and Pseudemys concinna. The nerves were dissected out as expeditely as possible and mounted in a nerve

[1] The facilities for carrying on this investigation were supplied by the Department of Physiology until these became available by the Department of Surgery.

[2] In this preliminary report, what is now termed the $B_1$ component was included in an $A$ process because of the similarities between the two components with respect to certain properties. It is now treated as a part of the $B$ elevation because with the distances of conduction available the component in question separates out from a first or $A$ component when this is present (for instance, in the cat vagus) more distinctly than it does from the following $B_2$. It might be noted here, however, that after conduction through the ganglion such separation is quite satisfactorily accomplished.

[3] This ganglion lies opposite one of the lowest cervical vertebra. It closely approximates the thoraco-abdominal trunk of the vagus, especially when the sympathetic and vagus nerves unite to form a common nerve trunk.

chamber supplied with oxygen throughout an experiment. Stimuli were applied either by means of induction shocks, galvanic currents or condenser charges (up to 160 volts and from 0.005 to 0.1 microfarad capacity with no difference in results. The latter type of stimulation has been found to be very satisfactory. The stimuli were applied through non-polarizable electrodes, the same type also serving as recording electrodes. The nerves were arranged in a bridge balanced for resistance and capacity as described by Bishop (1927). The recording mechanism was the cathode ray oscillograph in combination with an amplifier yielding amplification up to 200 millimeters per millivolt.

TABLE 1

*Threshold stimulating currents for $B_1$, $B_2$ and $C$ potential components in pre- and postganglionic stretches of nerve and through the cervical sympathetic ganglion*

| EXPERIMENT DATE | WAVE | SITE | | | |
|---|---|---|---|---|---|
| | | Preganglionic threshold | Postganglionic threshold | Efferent threshold | Through ganglion efferent threshold |
| November 4, 1929 | $B_1$ | 1.5– | 1.5 | 1.5 | 1.5 |
| | $B_2$ | 4.5 | 6.0 | | 4.5 |
| | $B_3$ | | | 12 | |
| | C | 28.5 | 31.5 | | 28.5 |
| September 30, 1929 | $B_1$ | 4.5– | 4.5 | | 4.5 |
| | C | 16.5–22.5 | 16.5–22.5 | | 16.5–22.5 |
| | $B_3$ | | | 10.5 | |

Threshold values expressed in volts with a condenser of 0.01 microfarad capacity. The potential divider permitted voltage changes of 1.5 volts up to 16.5 volts and thereafter changes by steps of 22.5 volts plus 1.5 volt fractions of the first 16.5. Consequently it was impossible to register with greater accuracy than above indicated the threshold of the $C$ group when it fell between 16.5 and 22.5 volts.

For the determination of the absolutely refractory period the potentials were recorded after conduction to allow separation out of the waves. Condenser charges were employed as stimuli. These condensers were connected through two contacts of the circuit breaker and charged in series with the bridge containing the nerve, the whole being balanced for capacity and resistance. To prevent the first condenser shunting the second, the circuit breaker was made to open the first circuit before the second was closed, the first condenser charging through the nerve circuit in this interval.

RESULTS. On stimulating the preganglionic portion of the nerve preparation mounted as shown in figure 1A and recording from the postganglionic, three potential waves, $B_1$, $B_2$ and $C$, are obtained in certain prepara-

tions and in others only two, $B_2$ and $C$. It is invariably found that three waves are obtained through the ganglion when both the preganglionic and the postganglionic portions of fresh preparations taken separately give rise to three potential waves. When these trunks alone give rise to only $B_2$ and $C$ potentials, of course, only two waves are found on conduction

TABLE 2

*Conduction rates in pre- and postganglionic stretches of nerve and conduction time when conduction occurs through the ganglion*

| EXPERIMENT | TEMPERATURE | WAVE | SITE | | | | CONDUCTION DISTANCE | COND. RATE M.P.S. | TOTAL COND. TIME, GANGLION AND SYNAPSE DELAY | SYNAPSE SIGMA |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | Preganglion | Postganglionic | Through ganglion: Afferent | Through ganglion: Efferent | | | | |
| | | | | | | | *mm.* | | | |
| February 14, 1929 | 21 | $B_1$ | | + | | | 30 | 8.7 | | |
| | | $B_2$ | | + | | | | 3.6 | | |
| | | $B_3$ | | + | | | | 1.5 | | |
| | | C | | + | | | | 0.5 | | |
| | | $B_1$ | | | + | | | 8.2 | | |
| | | $B_3$ | | | + | | | 1.4 | | |
| | | $B_1$ | | | | + | | Not measurable | | |
| | | $B_2$ | | | | + | | | 17.6 | 9.3 |
| | | C | | | | + | | | 85.7 | 20.5 |
| October 10, 1928 | 21.7 | $B_1$ | + | | | | 26 | 8.0 | | |
| | | $B_2$ | + | | | | | 3.9 | | |
| | | C | + | | | | | 0.9 | | |
| | | $B_1$ | | | | + | | 8.0 | | |
| | | $B_2$ | | | | + | | | 16.7 | 9.6 |
| | | C | | | | + | | | 52.4 | 23.5 |
| | | $B_1$ | | | + | | | 8.6 | | |
| | | $B_3$ | | | + | | | 1.1 | | |

Synapse time for a given component was computed by subtraction from total time elapsed the conduction time at the rate in the pre- or postganglionic nerve previously determined for the component having a corresponding threshold. The small $B_3$ wave has a conduction rate of 1 to 1.8 M.P.S.; it is usually difficult to measure precisely unless the record is diphasic, because of its low amplitude and its position in the falling phase of the previous larger component.

through the ganglion. It is important to note (table 1) that the thresholds for the action potentials of the $B_1$, $B_2$ and $C$ groups through the ganglion are similar to those for these groups pre- and postganglionically.

The form and area of the $B_2$ and $C$ potentials obtained when the postganglionic portions of nerves alone are studied are comparable to the form

and area of these potentials obtained on conduction through the ganglion except for changes due to changes in distance of conduction and differences

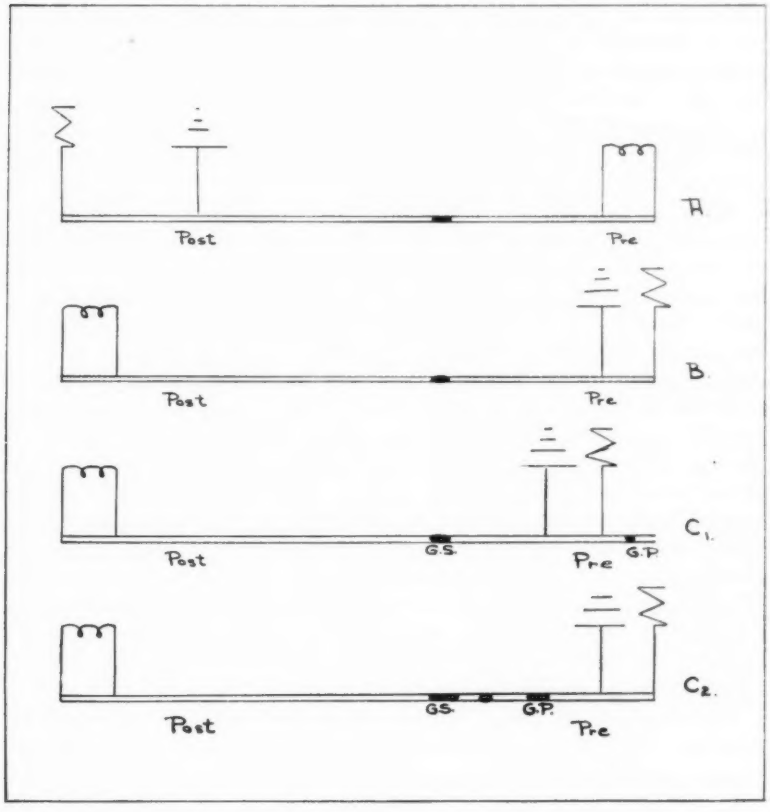


Fig. 1 illustrates the position in which the stimulating and recording electrodes are located on the nerve trunks.

*A*, where conduction is efferent through the superior cervical ganglion; *B*, where conduction is afferent through the superior cervical ganglion; $C_1$, where conduction is afferent through the superior cervical ganglion; and the recording electrodes distal to more proximal ganglia. Here an afferent $B_3$ wave is obtained; $C_2$, where conduction is afferent through the superior cervical ganglion and the recording leads distal to all the more proximal ganglia. Here no afferent $B_3$ wave has been obtained.

in conduction times of the $B_2$ and $C$ groups. The $B_1$ potential conducts without apparent delay through the ganglion in both afferent and efferent

directions (table 2). The areas of the $B_1$ potentials elicited separately pre- and postganglionically are of similar value in some nerves but in the majority the area obtainable postganglionically is considerably greater. The degree of difference is such as undoubtedly to be dependent upon an actual difference in the number of fibers. The difference in the relative areas occupied by the axons of the $B_1$, $B_2$ and $C$ fibers pre- and postganglionically does not seem adequate to explain the finding. Histological investigation indicates that there are frequently marked differences in the number of larger relatively thinly myelinated fibers pre- and postganglionically. The explanation for this difference is being sought for in a further investigation of the sensory components of the sympathetic and vagus nerve trunks of the neck and a study of the ganglionic cell types of these nerves. Branches leave the sympathetic trunks at various levels and these give histological evidence of having $B_1$ fibers mixed with $B_2$ and $C$ fiber types. It is also essential to note that the fibers responsible for the $B_1$ potential are readily injured by handling of the nerves, by anoxemia and by the application of stimuli of a strength adequate to elicit the $B_2$ and $C$ potentials. Errors due to these causes have been guarded against by careful dissection of the nerve, by supplying oxygen throughout the experiment and by confining the first observations of certain experiments to the first process. Because of the difficulties encountered in the study of the $B_1$ component in the sympathetic trunk it was deemed advisable to study the conduction of this group in another ganglion. It was possible without difficulty to demonstrate that this component conducted in both directions through the thoraco-abdominal ganglion of the turtle vagus without delay. This component is here a much more invariable part of the compound action potential than in the sympathetic trunk.

The $B_2$ and $C$ potentials are delayed in their conduction through the ganglion. This delay was measured by determining the conduction rates

---

Fig. 2. Strictly diagrammatic representation of the findings in the study of the conduction of the $B_1$, $B_2$ and $C$ potential components through the superior cervical ganglion of the turtle.

*1* and *2*. Typical conducted action potentials of pre- and postganglionic portions of the cervical sympathetic trunk; type I in which there is a $B_1$ component, type II in which there is no $B_1$ component.

*3* and *4*. Typical conducted action potentials through the superior cervical ganglion with the electrodes arranged as in *A*, figure 1, i.e., conduction efferently through the ganglion. In III a $B_1$ component is present, in IV only two potential components occur. $B_2$ may or may not be separate depending on the conducting distance.

*5* and *6*. Typical conducted action potentials through the superior cervical ganglion with the electrodes arranged as in *B* figure 1, i.e., conduction afferently through the ganglion; 5 shows the result when $B_1$ component is present, 6 when no $B_1$ component is present.

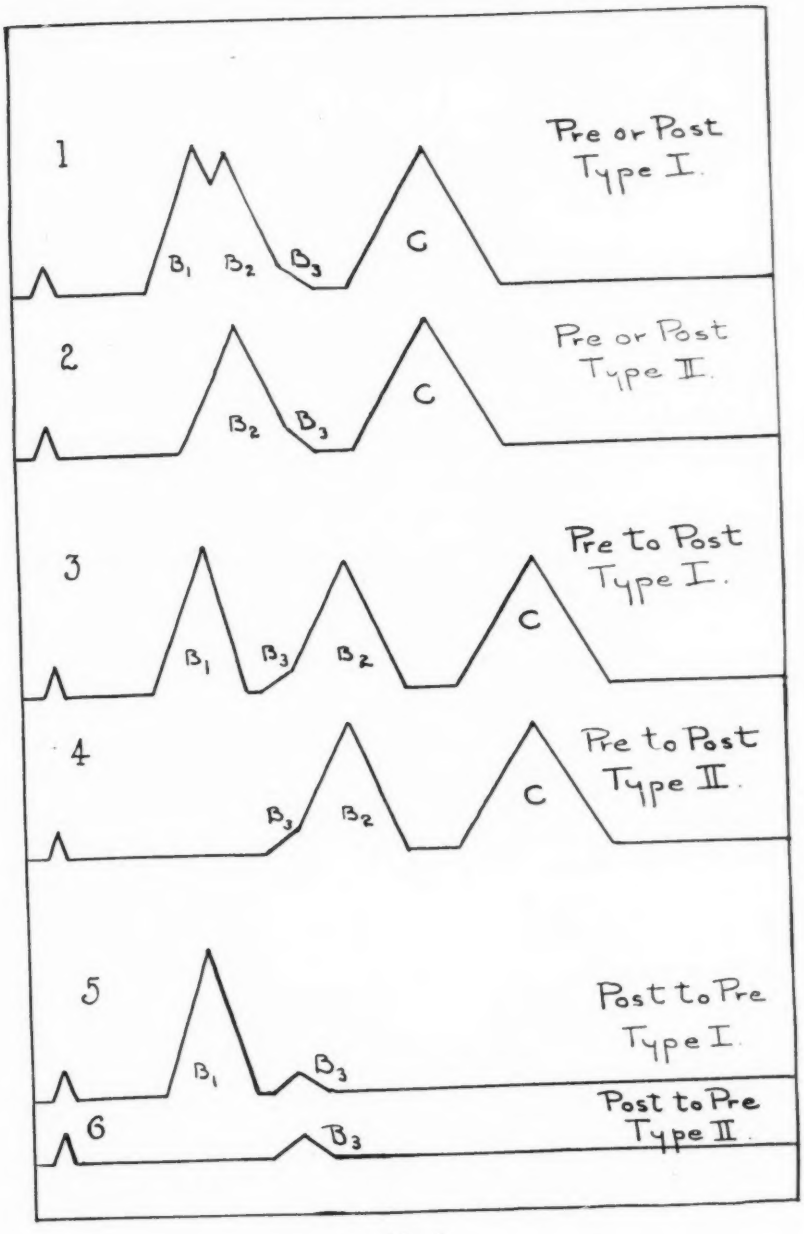
1
Pre or Post
Type I.
B1
B2
B3
C
2
Pre or Post
Type II.
B2
B3
C
3
Pre to Post
Type I.
B1
B3
B2
C
4
Pre to Post
Type II.
B3
B2
C
5
Post to Pre
Type I.
B1
B3
6
Post to Pre
Type II.
B3

Fig. 2

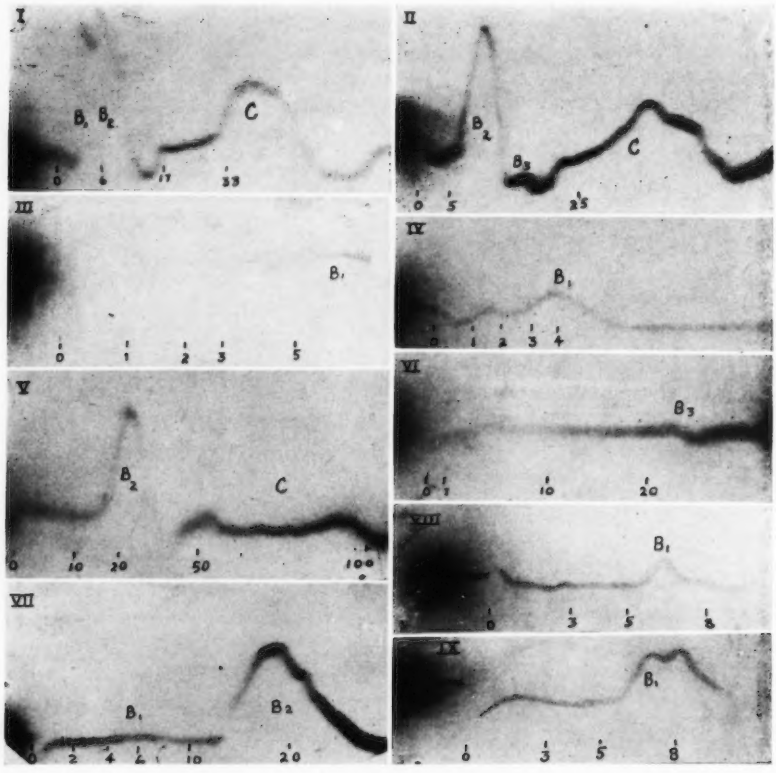


Fig. 3. Film I. Typical conducted action potential postganglionic portion of the cervical sympathetic trunk above the superior cervical ganglion. Three potential components are shown and named $B_1$, $B_2$ and $C$. Conduction distance 28 mm., temperature 24 degrees Centigrade. Time is marked in sigma in this and subsequent records.

Film II. Typical conducted action potential recorded from a similar portion of a sympathetic nerve trunk as in I in which only two potential components occur, $B_2$ and $C$, $B_3$ is considered part of the $B_2$ component. Conduction distance 20 mm., temperature 22 degrees Centigrade.

Film III. Conducted action potential through the superior cervical ganglion from the post- to the preganglionic portion, 41 mm. conduction. Only the first component is shown. Because of its threshold, conduction rate and absolutely refractory period it is considered similar to the $B$ process in somatic nerves. This potential conducts in both directions through the superior cervical ganglion without appreciable delay.

Film IV. Conducted action potential through the superior cervical ganglion from the pre- to the postganglionic region, 35 mm. conduction including the ganglion.

of these components in the pre- or postganglionic portions of the nerve trunk alone and then subtracting the time which would be necessary to travel the conduction distance at those rates from the time actually taken in travelling this distance when the conducting region included the ganglion. This average delay for the $B_2$ process in fresh preparations was 8 to 15 sigma and for the $C$ process 20 to 30 sigma. Determination of the absolutely refractory periods of these processes (table 3) through the ganglion yielded similar results. This indicates the reason for the delay and is also a simple method of determining synapse time.

On stimulating postganglionically with the recording leads on the preganglionic portion of the nerve (fig. 1B) there is generally obtained a small $B$ potential termed for convenience $B_3$. The area of this potential is only a relatively small fraction of the total area of the $B$ potential which conducts efferently through the ganglion. Its threshold and conduction rate are similar to the small wave following the main $B$ wave recorded in the potential of the postganglionic or preganglionic portions of the nerve alone. This small wave is described in greater detail in a paper presenting the analysis of the action potentials of the sympathetic and vagus nerves of the turtle and cat (Heinbecker, 1930). The small $B_3$ potential has so

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This process is similar in all its properties to that described in III. Because it conducts through the ganglion in both directions without delay and because its properties are similar to those of the main potential component in the turtle depressor nerve it is considered to arise in the sensory fibers of these nerve trunks.

Film V. Conducted action potential through the superior cervical ganglion from the pre- to the postganglionic portions of the nerve; 30 mm. conduction. Only two potential components $B_2$ and $C$ are present. Note the form of potential is similar to that shown in film II.

Film VI. Conducted action potential through the superior cervical ganglion from the post- to the preganglionic region (same preparation as in V); conduction distance 31 mm., conduction rate 1.4 m. p. s. This is the $B_3$ component referred to above, similar in properties to the $B_2$ component, but slower in conduction and higher in threshold. Its area is only a fraction of the total area of the $B$ elevation. It has never been found to conduct afferently beyond the most proximal ganglion on the nerve trunk.

Film VII. Conducted action potential from the pre- to the postganglionic region; conduction distance 21 mm. Note a low first component well separated from the second component because of the latter's delay in the ganglion.

Film VIII. Conducted action potential through the thoraco-abdominal ganglion of the vagus of the turtle from the pre- to the postganglionic region. Only the first component is shown, conduction distance 60 mm., conduction rate 6 meters per second. This potential has properties identical with those of the $B_1$ potential in the sympathetic nerves discussed in this paper.

Film IX. Conducted action potential from the post- to the preganglionic region of the same preparation as in VII; conduction distance 66 mm. Only the first component is considered here. Conduction rate 6.1 meters per second. The potential conducts through the ganglion in both directions with appreciable delay.

far never been found to conduct centripetally beyond the most proximal ganglion. This would indicate that when the potential is present on recording immediately proximal to the superior cervical ganglion it arises from fibers which have synapsed in the more proximal ganglia. Another proof that its conduction through the ganglion does not involve a synapse is the fact that its absolutely refractory period time through the ganglion varies from 4 to 5 sigma which is roughly the same value as at the stimulated point.

In this investigation no evidence of the presence of afferent *C* fibers has been obtained. If any *C* fibers synapse in the more proximal ganglia

TABLE 3

*Range of absolutely refractory periods of $B_1$, $B_2$, $B_3$ and C potential waves pre- and post-ganglionically and through the ganglion*

| WAVE | SITE | | | | ABS. REF. PERIOD SIGMA |
|---|---|---|---|---|---|
| | Preganglionic | Postganglionic | Through ganglion | | |
| | | | Afferent | Efferent | |
| $B_1$ | + | | | | 0.8 to 1.0 |
| $B_2$ | + | | | | 3.0 to 4.5 |
| C | + | | | | 3.5 to 6.0 |
| $B_1$ | | + | | | 0.8 to 1.0 |
| $B_1$ | | + | | | 3.0 to 5.0 |
| C | | + | | | 3.5 to 6.0 |
| $B_1$ | | | + | | 0.8 to 2.0 |
| $B_1$ | | | | + | 0.8 to 1.5 |
| $B_3$ | + | + | + | | 3.5 to 6 |
| $B_1$ | | | | + | 0.8 to 1.0 |
| $B_2$ | | | | + | 8 to 15 |
| C | | | | + | 15 to 30 |

An absolutely refractory period of 2 sigma indicates that there is depression or fatigue in the fiber. The $B_1$ fibers are with difficulty kept in good condition. The presence or absence of sensory ganglion cells has not been determined by this research.

their number is too small to exhibit a recognizable potential conducting afferently through the ganglion. At the amplification used (200 mm. per millivolt) a very small number of fibers should give a detectable potential.

To differentiate between the different components of the potentials, thresholds, conduction rate, chronaxie and absolutely refractory period times have been used as criteria (Heinbecker and Bishop, 1929, loc. cit.). Determination of the absolutely refractory period of the $B_1$ component through the ganglion yields a value similar to that of the fiber at the stimulated point when the preparation is fresh. At other times the value obtained may be twice the normal especially when the small preganglionic

trunk is stimulated and the recorded electrodes are on the postganglionic portion. At present it is considered that these differences are possibly the result of differential depression of the nerve axons in these two portions of the nerve trunk. This subject has been further investigated in the nerve roots and nerve trunks of the frog. Unless such preparations are extremely fresh or preserved in cold Ringer's solution the absolutely refractory period times of axons giving rise to Alpha potential wave in the roots may be 1.5 to 2 times those of the same axons in the nerve trunk. These findings apply to both motor and sensory roots and are apparently not causally related to the passage of the potential through the ganglion.

The $B_1$ potential is seen to differ markedly in its properties from both the $B_2$ and $C$ potentials. Because of its variable presence and the fact that it conducts in both directions without synaptic delay in its passage through the ganglion it is considered to arise from afferent fibers of the nerve trunk.[4] The previous evidence which indicated that this potential arises from the larger relatively thinly myelinated fibers (also variably present), substantiates this conclusion. Histologically Kiss and Mihalik (1928) have presented evidence that certain fibers of somatic nerves characterized by a thin myelin sheath are sensory. Further convincing evidence is obtained by a study of the depresser nerve of the turtle, cat and rabbit (Bishop and Heinbecker, 1930). The most rapidly conducting fibers of these nerves give rise to a potential having the physiological properties of the $A$ or $B_1$ potential, and they possess the histological picture of fibers responsible for the $B_1$ component in autonomic nerve trunks.

The $B_2$ potential in these nerves arises from the smaller thinly myelinated fibers which synapse in the superior cervical ganglion. On the basis of threshold, conduction rate and form of potential it is apparent that the physiological and histological characteristics of the fibers are the same on either side the synapse. Convincing proof of the similarity of the $A$, $B$ and $C$ potentials pre- and postganglionically and through the ganglion is obtained in their corresponding threshold values. It is also possible to determine the conduction rates of the $B_2$ and $C$ potentials for two different conducting distances after conducting through the ganglion. The difference in conduction time for the $B_2$ and $C$ potentials permits the calculation of the conduction rate of these potentials in a given stretch of nerve trunk in which the ganglion is not concerned. The con-

[4] This reversible passage through a sympathetic ganglion does not of course prove that these fibers are sensory. Absolute proof of the afferent character of fibers with the same set of properties in the vagus has been secured in a study of ganglia by the author and Dr. James O'Leary (unpublished) where the cell bodies of these fibers can be identified by the effects of differential stimulation of the separate fiber group. In the gray ramus of the dog where afferent fibers pass into the sciatic nerve from the sympathetic chain a $B_1$ potential may also be identified as arising from them (Bishop and Heinbecker 1930).

duction rates show that the $B_2$ potential travels 3.5 to 3 M per second and the $C$ potential travels 0.8 to 0.3 M per second. These are the rates for the $B$ and $C$ potentials in these nerves when no synapse is involved. A typical experiment is cited.

| | |
|---|---|
| I. Conducting distance including the ganglion | 42 mm. |
| Conduction time $B_2$ wave | 36 sigma |
| Conduction time $C$ wave | 142 sigma |
| II. Conduction distance including the ganglion | 28 mm. |
| Conduction time $B_2$ wave | 32 sigma |
| Conduction time $C$ wave | 104 sigma |
| Conduction time for $B_2$ wave to travel 14 mm. in nerve trunk | 4 sigma |
| Conduction time for $C$ wave to travel 14 mm. in nerve trunk | 38 sigma |
| Conduction rate of $B_2$ wave | 3.5 M per second |
| Conduction rate of $C$ wave | 0.36 M per second |
| Delay of $B$ in ganglion | 24 sigma |
| Delay of $C$ in ganglion | 26 sigma |

There is here no evidence that postganglionically there is a loss of myelin sheath; the $B_2$ potential presumably always arises from small thinly myelinated fibers in these nerves both pre- and postganglionically (Heinbecker, 1930). The experiment further illustrates fatigue of the $B$ synapse because of rather prolonged stimulation ($C$ synapse was not stimulated as long) at a rate, however, not rapid enough to fatigue the $B$ fiber as evidenced by its normal conduction rate. In fresh preparations as stated about the $B$ synapse time averages 8 to 15 sigma.

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Fig. 4. *1.* Photomicrograph of preganglionic (with reference to the superior cervical ganglion) portion of the cervical sympathetic trunk in the turtle showing three fiber types and giving rise to three potential components named respectively $B_1$, $B_2$ and $C$. Typical fibers are marked on the photomicrograph.

*2.* Photomicrograph of postganglionic portion of the same nerve as shown in *1.* It also is composed of three types of nerve fibers.

*3.* Photomicrograph of preganglionic portion of cervical sympathetic trunk in which almost all the fibers belong to the second and third types, i.e., give rise to the $B_2$ and $C$ potentials, and no $B_1$ component was demonstrable.

*4.* Photomicrograph postganglionic portion of same nerve as shown in *3.* Here again the fibers are largely, though perhaps not exclusively, of the second and third types. However, stimulation of the nerve preganglionically resulted in no detectable $B_1$ potential postganglionically, because the preganglionic nerve III had even fewer if any $B_1$ fibers than the post.

*5.* Photomicrograph postganglionic portion of cervical sympathetic trunk showing three types of fibers giving rise to the $B_1$, $B_2$ and C potentials.

*6.* Photomicrograph postganglionic portion of cervical sympathetic trunk showing only two types of fibers, those giving rise to potential $B_2$ and $C$. Magnification 1, 2, 3 and 4, 5 and 6, one thousand times. All nerve preparations treated in the same manner, i.e., 1 per cent osmic acid for 24 hours.

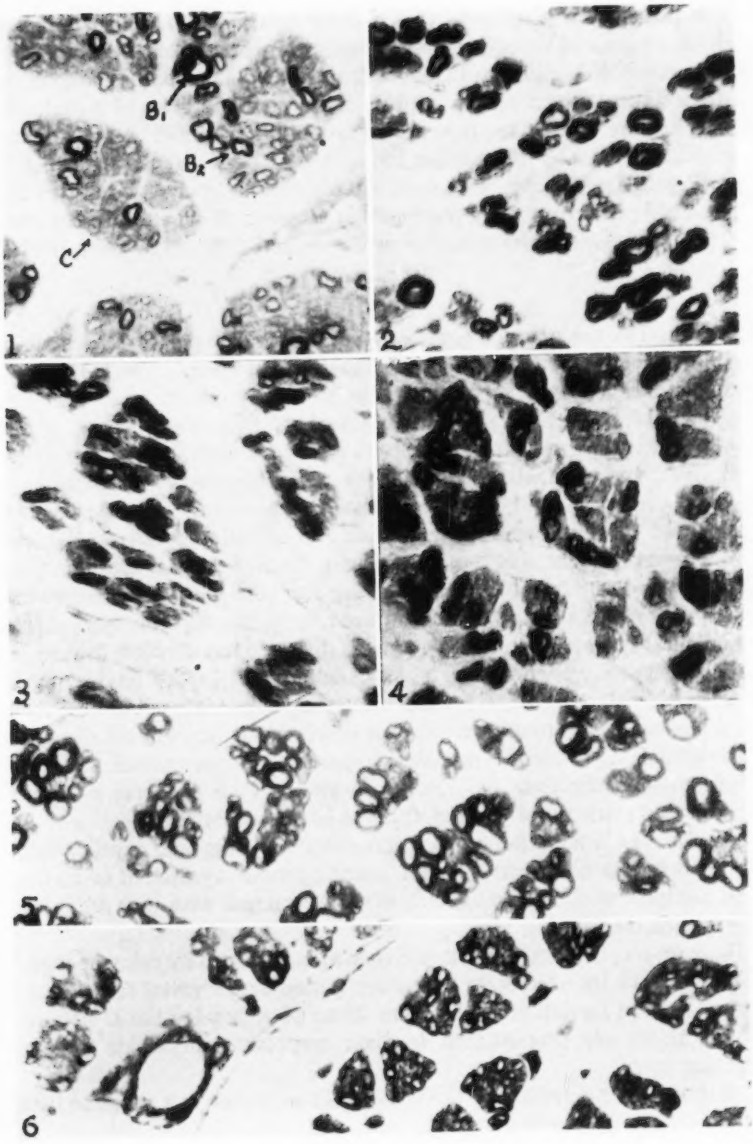


Fig. 4

The presence of a synapse is ordinarily indicated by the inability to conduct a potential through it retrogradely. It is possible to demonstrate the presence of a synapse in another manner. Pure carbon dioxide of itself has been shown not to depress the action potential of a normally active fiber of the $A$ component to extinction (Heinbecker, 1929), and unpublished data by the author indicate that this also holds for the $B$ and $C$ groups. If a synapse occurs the conduction of a potential through it is quickly interrupted by pure carbon dioxide. This method has been used to demonstrate the synapses of the $B_2$ and $C$ fibers in these nerves.

## SUMMARY

Conduction through the cervical sympathetic ganglia of the turtle of the fibers types present in this nerve has been studied. The nerve lacks the first component of fibers characteristic of somatic nerves. The first or $B_1$ potential of this nerve when present pre- and postganglionically conducts through the ganglion in both directions without apparent delay. This fact, together with the evidence that the fibers giving rise to it have physiological and histological characteristics similar to those of fibers considered to be afferent in autonomic and somatic nerve trunks, seems to warrant the conclusion that the fibers of the $B_1$ potential are afferent.

The major portion of the fibers giving rise to the $B_2$ potential and all fibers giving rise to the $C$ potential synapse in the superior sympathetic ganglion. The action potentials of such fibers do not conduct toward the central nervous system through the ganglion. In turtles having one or more proximal ganglia besides the customary superior a small number of $B_2$ fibers conduct through the superior cervical ganglion in both directions without delay. These fibers have synapsed in the more proximal ganglia. Postganglionically they conduct more slowly than the rest of the $B_2$ fibers. No evidence of afferent $C$ fibers has been obtained.

In the turtle superior cervical ganglion, preganglionic myelinated or $B_2$ fibers are found to synapse with postganglionic myelinated or $B_2$ fibers and unmyelinated $C$ preganglionic fibers to synapse with unmyelinated $C$ postganglionic fibers.

In fresh preparations the synapse time as determined by delay in conduction rate and by absolutely refractory period times varies from 8 to 15 sigma for the $B_2$ potential and from 20 to 30 sigma for the $C$ potential. These times are proportional to their respective thresholds and conduction rates.

A method for determining the presence of a synapse in a ganglion by the use of carbon dioxide is described.

It is a pleasure to acknowledge the interest of Dr. George H. Bishop in this work.

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# PHYSIOLOGY OF THE SPHENOPALATINE GANGLION

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This investigation was undertaken in the hope of clearing up the confusion that now exists in regard to the vasomotor and sensory innervation of the nasal mucous membrane.

The sphenopalatine ganglion has been the object of attention of some oto-laryngologists for many years who attempt to relieve through it the pain associated with various conditions of the nose, throat, eyes, sinuses, and, in fact, structures quite remote from the head. Foremost in this work was Sluder (9), who in his book states that by the application of alcohol or cocaine to the sphenopalatine ganglion, the pain of many conditions of the head is mitigated or completely stopped. The effect of such therapy may last for days or months, depending on the severity of the condition, and the individual himself. A voluminous bibliography is included in Sluder's monograph.

Burger (2) reviews the literature quite extensively and attempts to correlate the clinical findings with what is known of the physiology of the sphenopalatine ganglion. While he agrees with Sluder, he is inclined to be more conservative in his statements.

One is impressed with the great quantity of clinical literature as well as with the paucity of available physiological data. Müller (7) has very little to say on the function of the ganglion and its connections. The recent German work "Handbuch der Normalen und Pathologischen Physiologie" is equally barren on the subject. However, several productive researches have been reported, and they will be mentioned in the discussion.

The sphenopalatine ganglion belongs to the system of outlying sympathetic ganglia, in which are included, in addition, the otic, optic and submaxillary. In the dog the ganglion presents itself as a swelling on the Vidian nerve, and is attached anteriorly to the posterior nasal nerve. The nerve fibers of the ganglion all join the posterior nasal nerve of the trigeminus, and are distributed with this nerve to the middle and inferior turbinates, and the lower part of the nasal septum and lateral nasal wall. The Vidian nerve is made up of the greater superficial petrosal nerve of the

facial nerve and the deep petrosal nerve of the internal carotid plexus (fig. 1).

The remainder of the nasal mucous membrane is supplied by the nasociliary branch of the ophthalmic nerve, but this is only a small portion of the entire mucous membrane.

METHODS. The vasomotor reactions in the nasal mucosa were measured by the method described by Tschallussow (10), which depends upon the change in volume of the nasal cavity produced by the vasomotor activities.

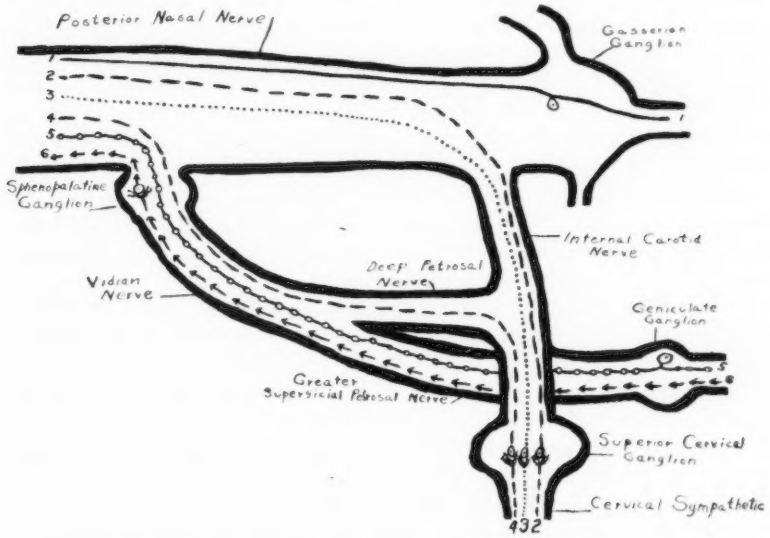


Fig. 1. Diagrammatic representation of the vasomotor and sensory innervation of the nasal mucous membrane of the dog.

*1.* Somatic sensory fiber of the posterior nasal nerve. *2.* Vaso-constrictor fiber of the posterior nasal nerve. *3.* Vaso-dilator fiber of the cervical sympathetic. *4.* Vaso-constrictor fiber of the Vidian nerve. *5.* Visceral sensory fiber of the Vidian nerve. *6.* Vaso-dilator fiber of the Vidian nerve.

An incision is made in the median raphe of the soft palate extending two centimeters back of the hard palate. A wad of cotton saturated with vaseline is forced through the opening and into the posterior nares, sealing it completely. A glass tube is inserted into one anterior nares, and sealed with vaseline and cotton. In this way one side of the nose is transformed into an airtight cavity, and any change in nasal volume is recorded by a tambour which is connected to the glass tube in the nose. A vaso-constriction results in a decrease in nasal volume and the tambour lever drops.

A vaso-dilatation causes the elevation of the tambour. The incisive foramen need not be plugged because no air escapes through it.

The ganglion is approached as follows: An incision is made from the outer angle of the eye to the ear. The skin is reflected from the masseter muscle and it is removed. The zyomatic arch is freed from muscle and resected. This exposes the coronoid process of the mandible, on which the temporal muscle is inserted. The temporal muscle is freed, and the coronoid process removed. The external pterygoid is thus brought into view, and by blunt dissection its fibers are carefully pulled away, exposing the lachrymal gland. The gland is reflected to one side, and this exposes the maxillary division of the trigeminal nerve. The uppermost bundle of fibers are quite distinct, and make up the infra-orbital nerve, which passes forward to the skin of the face under the eye. This nerve is reflected and cut, which exposes the posterior nasal nerve and the sphenopalatine ganglion. The ganglion appears as a long drawn out bluish grey structure attached to the posterior nasal nerve by its anterior end. At its posterior end it narrows down to a fine nerve, the Vidian, which, if traced farther back, can be seen to divide into its two component branches, the greater superficial petrosal, and the deep petrosal.

In these experiments dogs under barbital anesthesia were used. Blood pressure was recorded by a manometer from the femoral artery and respiration by pneumograph and tambour.

Results. *Vasomotor pathways to the nasal mucous membrane.* Stimulation of the central end of the vago sympathetic trunk with a weak faradic current results in a vaso-constriction of the blood vessels of the nasal mucous membrane on the same side of the head. The nasal chamber on the other side is unaffected. Stimulation of the posterior nasal nerve central to the sphenopalatine ganglion also results in a vaso-constriction, but to a lesser extent. The infra-orbital nerve does not carry vasomotor fibers for the nasal mucous membrane. Stimulation of the peripheral end of the Vidian nerve results in a vasodilatation of the blood vessels of the nasal mucous membrane on the same side of the head.

One per cent nicotine applied to the superior cervical ganglion produced a marked but transitory vaso-constriction in the nasal mucous membrane. Stimulation of the central end of the vago sympathetic trunk after the stimulating effect of the nicotine had worn off resulted in a very slight vaso-constriction. We were never able to produce a complete block. Nicotine applied to the sphenopalatine ganglion had no effect on the vaso-constriction produced by the stimulation of the posterior nasal nerve.

The purely vaso-constrictor effect from posterior nasal nerve stimulation, and the purely vaso-dilator effects of Vidian nerve stimulation may mean merely that these systems predominate in the respective nerves. In order

to determine whether vaso-dilators are present in the posterior nasal, and constrictors in the Vidian, the following experiments were performed.

In four dogs the superior cervical ganglion was removed. In two, stimulation of the posterior nasal nerve fourteen days after the operation resulted in a vasodilatation that was not abolished by nicotine applied to the sphenopalatine ganglion. The two other dogs were used eighteen days after the operation, and in these, posterior nasal stimulation was entirely without effect.

As mentioned above, stimulation of the Vidian nerve produces a vasodilatation in the nasal mucous membrane. Nicotine applied to the sphenopalatine ganglion produces a transitory vasodilatation. Stimulation of the Vidian nerve after nicotine has acted now produces a vaso-constriction instead of a vasodilatation. In those dogs used eighteen days after their superior cervical ganglia had been removed the vaso-constrictor effects of the Vidian nerve after nicotine application to the sphenopalatine ganglion were entirely absent.

*These experiments indicate that both the posterior nasal and Vidian nerves carry constrictor fibers for the nasal mucous membrane and both carry dilators for the nose also.*

2. *Sensory nerves to the nasal mucous membrane.* Stimulation of the central end of the Vidian nerve with a weak faradic current produces pronounced reflex effects. During the stimulation the mouth is opened widely, the tongue protruded, the head flexed downwards on the neck, and the eyes are deviated markedly to one side or the other. Each of these effects is extremely powerful and brisk and persists as long as stimulation is continued.

In addition, marked respiratory and circulatory effects are evoked. Respiration is increased very decidedly, both in rate and depth, in a manner equal to that obtained from stimulation of the central end of the sciatic nerve. The blood pressure is lowered appreciably (fig. 2).

Stimulation of the central end of the posterior nasal nerve results in essentially the same effects, but to a lesser extent.

Since the Vidian nerve is composed of two components—the greater superficial petrosal and the deep petrosal of the sympathetics an attempt was made to determine which of these two nerves carried the afferent impulses, or if both carried them. After the above mentioned reflexes obtained, the homolateral vagus nerve (which carries the cervical sympathetic) was sectioned, and the Vidian nerve was again stimulated. The results were identical with those obtained before vagotomy, indicating that the sensory fibers belong to the greater superficial petrosal nerve. The peripheral end of the vagus nerve was stimulated after the administration of one-half milligram of atropine, and no changes were produced on the blood pressure and respiration. In this case the central end of the sympa-

thetic is stimulated, and if the afferent impulses followed the sympathetic, the respiratory and hemodynamic reflexes should be duplicated by stimulation of the sympathetic. This constitutes another proof that the sensory fibers do not run in the vago-sympathetic trunk.

The vagal inhibition of the heart accounts for the hemodynamic response. After one-half milligram of atropine was given intravenously to

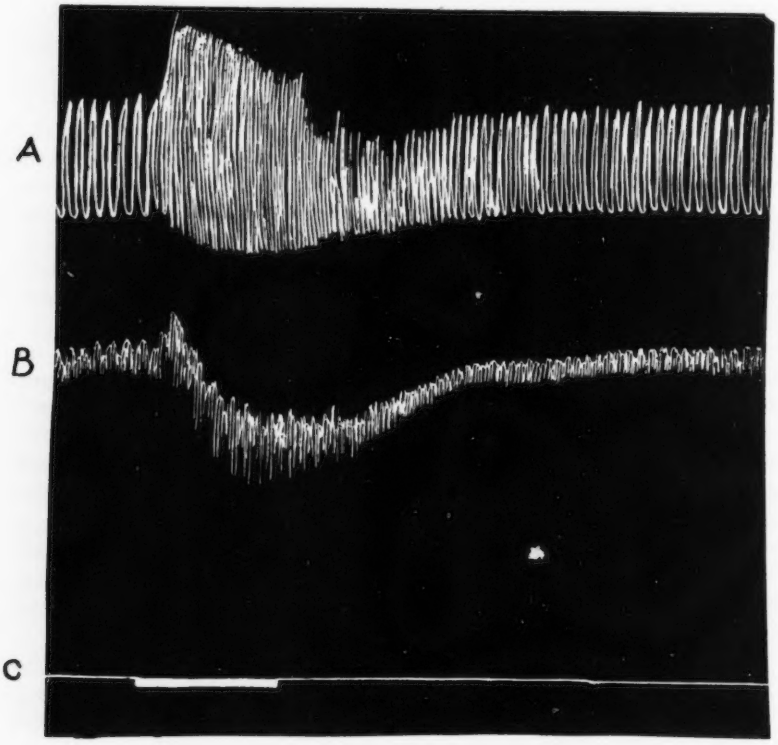


Fig. 2. Effects of stimulation of the central end of the Vidian nerve. *A*. Respiration. *B*. Blood pressure. *C*. Stimulation for 10 seconds.

block the inhibitory fibers to the heart, the blood pressure depression was practically nil, except for a slight lowering that may be accounted for by the respiratory acceleration.

In addition to the above reflexes there are produced reflex effects on the nasal mucous membrane itself. By grading the depth of the anesthetic, the hemodynamic reflexes could be eliminated, but stimulation of the

central end of either the Vidian or posterior nasal nerves results in a reflex vaso-constriction in the contralateral nasal chamber, which is greater in the case of the Vidian nerve. The vagus nerve was now sectioned on the stimulated side and the reflexes were obtained as before, ruling out the cervical sympathetic as the nerve concerned in carrying the afferent side of the reflex. When the cervical sympathetic was cut on the side on which the vasomotor reflexes were obtained, no response was possible, indicating that the efferent path was through the cervical sympathetic. In no case was it possible to obtain vaso-dilator reflexes from Vidian or posterior nasal stimulation.

Vasomotor reflexes to the nose were obtained also by stimulation of the central end of the sciatic nerve. Both vaso-dilator and vaso-constrictor reflexes were obtained by grading the strength of the stimulating current. Stretching the omentum, manipulation of the subcutaneous tissue of a skin incision, and stimulation of the central end of the contralateral vagus nerve, were all productive of a vasomotor change in the nose. All the effects were abolished when the vagus was sectioned on the side that was being recorded. In contrast to the reflexes obtained from the Vidian and posterior nasal nerves, no vasomotor change could be elicited in the nose from sciatic, skin and omental stimulation except when a change was obtained in the general blood pressure, that is, a rise in blood pressure was accompanied by a constriction in the nose, and a drop in pressure by a dilatation.

Discussion. *Vasomotor pathways.* From the above results, the vaso-constrictors reach the nose as follows: from the superior cervical ganglion they travel to the internal carotid nerve; the major portion of them reach the posterior nasal nerve. A lesser number pass by way of the deep petrosal from the internal carotid nerve to the Vidian nerve, traverse the sphenopalatine ganglion, and reach the nose by way of the posterior nasal nerve. The connection between the posterior nasal nerve and the internal carotid nerve probably occurs in the cavernous sinus where the sympathetic plexus makes many connections with the branches of the trigeminus.

Our results indicate that most of the constrictors undergo a synapse in the superior cervical ganglion. Possibly the reason why a complete block to the fibers could not be obtained is because the superior cervical ganglion is invested in a tough fibrous capsule which might prevent thorough infiltration of nicotine solution through it.

There is certainly no synapse for the constrictors in the sphenopalatine ganglion. Nicotine produces no block when it is applied. Teshallusow (10), working on this problem on the dog, reports by use of nicotine and degeneration methods that the vaso-constrictors do not synapse in the superior cervical ganglion, but in the sphenopalatine ganglion. Our results do not support his findings.

When the posterior nasal nerve was stimulated fourteen days after the removal of the sphenopalatine ganglion, a vaso-dilatation was obtained. Nicotine on the sphenopalatine ganglion failed to abolish this result. We are then dealing with vaso-dilators from the cervical sympathetic which probably synapse in the superior cervical ganglion, and pass by way of the posterior nasal nerve to the nose, uninterrupted in the sphenopalatine ganglion. Eighteen days after removal of the ganglion, the posterior nasal nerve possesses no vasomotor fibers because they have entirely degenerated. It is only because the constrictor fibers degenerate more quickly that we can demonstrate the dilators by this method. The literature is conflicting on the presence of dilators in the cervical sympathetic nerve. Dastre and Morat (3) obtained vaso-dilatation of the lips, gums, and cheeks by stimulating the white rami of the second to fifth thoracic nerves. Isergin (5) demonstrated vaso-dilators to the buccal mucosa by weak stimulation of the neck sympathetic. Still others report they were unable to prove vaso-dilators in the cervical sympathetic. By the methods here presented, there are unmistakably dilators to the nasal mucous membrane in the cervical sympathetic, and they follow the same path as that taken by the constrictors.

Tschallusow (10) was the first to demonstrate the vaso-dilator action of the Vidian nerve. The dilator fibers of this nerve synapse in the sphenopalatine ganglion and pass by the posterior nasal nerve to the blood vessels of the nose. Nicotine blocks these fibers absolutely when it is applied to the sphenopalatine ganglion. Larsell and Fenton (6), in an anatomical study, describe small fibers with myelin sheaths which run in the greater superficial petrosal nerve, and state that they are of a preganglionic type. It is entirely possible that these are the preganglionic vaso-dilators, but, of course, it is equally possible that they are the preganglionic fibers with secretory function about which so little is known.

Since the posterior nasal nerve possesses no vasomotor action eighteen days after the removal of the superior cervical ganglion, we have reached the conclusion that this nerve carries no antidromic vaso-dilators. If it did they should be demonstrable even after the superior cervical ganglion is removed.

*Sensory nerves from the nose.* The sensory nerves of the nose are the posterior nasal branch of the maxillary division of the trigeminus, the greater superficial petrosal of the facial and the naso-ciliary branch of the ophthalmic division of the trigeminus. The two nerves studied—the posterior nasal and the Vidian—are capable of producing extremely powerful reflexes. This is especially surprising in the case of the Vidian which is hardly larger in diameter than a silk thread, and yet its effects upon respiration are fully as profound as those produced by the sciatic nerve (fig. 2).

As mentioned above, stimulation of these nerves produces reflex effects upon the head, eyes, neck, tongue, respiration and heart. In the case of the Vidian nerve, the afferent fibers run in the greater superficial petrosal, and not the sympathetic, because section of the sympathetic on the same side does not abolish the reflexes. The sensory character of the greater superficial petrosal has been hinted at by two workers. Yagita (11) cut this nerve and demonstrated chromatolysis in the cells of the geniculate ganglion. Larsell and Fenton (6) were able to show anatomically large sensory fibers in the greater superficial petrosal nerve, which in all probabilities are those we stimulated, and are responsible for the sensory character of the greater superficial petrosal nerve. They have demonstrated unipolar cells of the sensory type in the geniculate ganglion, and regard it as a member of the cranio-spinal series of sensory ganglia.

The hyperpnea produced by stimulation of the posterior nasal and Vidian nerves is quite unique when one considers that in general stimulation of the sensory nerves of the respiratory passages produces inhibition of respiration. The Vidian nerve is a visceral afferent and the posterior nasal a somatic afferent, yet both produce the same hyperpnea. This does not preclude the possibility of inhibiting fibers from the nose because Falk (4) has demonstrated in the dog that water on the nasal mucous membrane will produce an inhibition of respiration.

Our results indicate that no sensory fibers are present in the cervical sympathetic in the dog. Ranson and Billingsley (8) have come to the same conclusion in the cat.

These findings in the dog furnish but little help toward explaining the clinical results reported as following cocainization of the sphenopalatine ganglion.

## CONCLUSIONS

1. In the dog vaso-constrictor fibers for the nasal mucous membrane travel as follows: the preganglionic fibers run in the cervical sympathetic and synapse in the superior cervical ganglion. The post-ganglionic fibers go to the internal carotid nerve; the major portion of them reach the posterior nasal nerve and reach the nasal mucous membrane with this nerve. A small number of fibers reach the deep petrosal, go to the Vidian nerve, traverse the sphenopalatine ganglion, and reach the nose by way of the posterior nasal nerve.

2. In the dog vaso-dilator fibers for the nasal mucous membrane travel in two ways: *a*. From the facial nerve the preganglionic fibers travel in the major superficial petrosal to the sphenopalatine ganglion, where they undergo a synapse. The post-ganglionic fibers enter the posterior nasal nerve and go to the nose. *b*. Vaso-dilators run in the cervical sympathetic and probably synapse in the superior cervical ganglion. From here they

travel in the internal carotid nerve, to the posterior nasal nerve, and reach the nasal mucous membrane.

3. No antidromic vaso-dilators are present in the posterior nasal nerve of the dog.

4. Sensory nerves for the nasal mucous membrane are visceral and somatic. The visceral fibers run through the sphenopalatine ganglion to the Vidian nerve to the greater superficial petrosal nerve to the facial nerve. The somatic fibers run in the posterior nasa nerve of the trigeminus. Both sets of fibers are capable of producing respiratory, cardiac, ocular, facial, and lingual reflexes.

5. Reflex vasomotor changes in the nasal mucous membrane can be evoked by stimulation of the Vidian nerve, posterior nasal nerve and others.

6. There are no sensory fibers in the cervical sympathetic of the dog.

7. These findings on the dog do not seem to help in the explanation of effects reported from cocainizing the sphenopalatine ganglion in man.

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# THE FATAL EFFECT OF THE TOTAL LOSS OF GASTRIC JUICE[1]

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A more or less complete loss of gastric juice from the body occurs in cases of clinical or experimentally produced obstruction at the pylorus of the stomach or in the upper small intestine. Little or no absorption takes place in the stomach or duodenum and the gastric juice secreted is lost through vomiting. It has been demonstrated by MacCallum and his associates (1920), Hastings, Murray, and Murray (1921), Haden and Orr (1923), and Gamble and Ross (1925) and others that when the pylorus is completely obstructed in experimental animals, death occurs within three or four days and the blood shows certain definite and characteristic chemical changes. There is a profound decrease in the concentration of chloride ion, a simultaneous increase in $CO_2$ capacity and later an increase in non-protein and urea nitrogen. Among the possible interpretations of these effects, two have received widest attention. It has been suggested that a toxemia developes that withdraws chloride from the blood perhaps through a mechanism similar to that occurring in pneumonia and if not promptly relieved causes death. On the other hand the changes in the blood and subsequent morbidity and death have all been attributed to the failure of reabsorption of water and salts excreted in the gastric juice. Such a theory necessarily assumes that the gastric glands can continue to excrete in the gastric juice chloride and perhaps other inorganic ions when the concentration of these substances in the blood has been reduced below that normally present. The present experiments were devised to test this possibility.

It was desirable to provide a method permitting the complete loss of gastric juice from the body under conditions otherwise as nearly normal as possible and without involving the element of obstruction anywhere in the gastro-intestinal tract. Recourse was made to a method described by Lim, Ivy, and McCarthy (1925) with this important modification that the

[1] A preliminary report of this work was published in the Proc. Soc. for Exp. Biol. and Med., 1929, xxvi, pp. 305-307, and a second report read at the XIIIth International Physiological Congress. (This work has been conducted under a grant from the Douglas Smith Foundation for Medical Research of the University of Chicago.)

vagus nerves to the isolated stomach were left largely intact. Under ether anesthesia the stomach was cut across at the cardia and the pylorus. The duodenum was anastomosed to the lower end of the esophagus. The cardiac end of the isolated stomach was infolded and closed and the pyloric end brought to the surface as a fistula. A special gold plated cannula was used for this purpose and by this means it was possible to collect the gastric secretion without loss through leakage or corrosion of the surrounding skin.

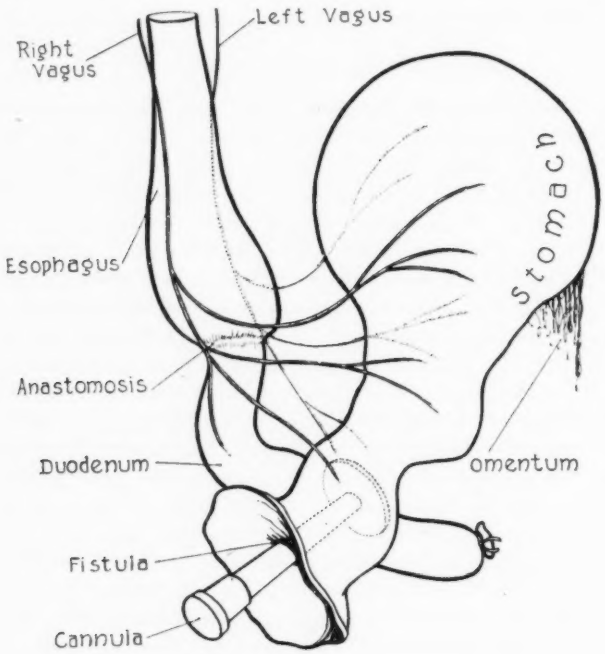


Fig. 1

Great care was observed during the operation not to interfere with the vagus nerves or blood vessels supplying the isolated stomach. In each case the entire left vagus was preserved and a majority of the gastric fibers of the right vagus. Occasionally fibers from the right vagus passing along the anterior surface of the lower esophagus had to be sacrificed to make the anastomosis. The blood supply to the stomach was not interfered with except for the small portion supplied by the vessels at the pylorus. Figure 1 illustrates the preparation. Six animals survived the operation and in these daily observations were made on the amount and quality of gastric secretion, food and water intake, urinary excretion, and blood chemistry. A part of

these data have been considered adequate for the purpose of this paper. The following points have been established.

1. *The total loss of the gastric secretion from the body causes death in five to eight days.* In the case of the six animals in which there was no technical fault the post-operative course was similar. Recovery from the immediate effects of the operation was rapid and within 24 hours the animal appeared quite lively. Five hundred grams of ground meat, 500 cc. of milk, and 2 to 3 liters of water were supplied daily after the first 48 hours. At first the meat was taken voraciously only to be promptly regurgitated. The animals however soon learned and thereafter took both the meat and fluids in small amounts at short intervals during the entire 24 hours. Perhaps for this reason greater quantities of gastric juice were secured at night than during the day.

In two cases no therapy of any kind was used and these animals lived seven and eight days respectively. In the case of the other four, as as soon as the condition of the animal became so precarious that it was readily apparent that it would not survive the day, treatment was instituted and life prolonged. The symptoms observed in the order of their appearance were loss of weight, anorexia, decreased urinary secretion, muscular weakness, and profound depression. The loss of weight frequently amounted to from 0.5 to 1.5 kgm. per 24 hours. The amount of food and water taken gradually decreased until none whatever was taken during the last 2 days. The suppression of urinary secretion followed pari passu with the decreased water intake. It is interesting, in view of the marked hypo-chloremia and alkalosis, that no symptoms of tetany or neuro-muscular hyper-excitability were observed although carefully watched for. In each case the deviation from the normal was in the direction of depression. The temperature likewise remained within normal limits.

2. *The continued loss of gastric juice from the body produces changes in the blood chemistry similar to those occurring after pyloric obstruction.* These changes are illustrated in tables 1, 2 and 3. There occurs a prompt and progressive decrease in the concentration of blood chloride (from a normal of 300 to 108 mgm. of chloride ion per 100 cc.), an increase in the carbon dioxide capacity (from a normal of 45 to 140 cc. per 100 cc.), a shift in the pH toward the alkaline side (from a normal of pH 7.30 to 7.75), and a terminal increase in the non-protein nitrogen (from 27 to 180 mgm.) and urea nitrogen (from 12 to 154 mgm.). The changes in blood chemistry accompany the symptoms of depression, etc., and are roughly proportionate to their severity. A number of determinations of blood volume have demonstrated no decrease produced by the loss of gastric juice, and estimations of hemoglobin, number of red blood corpuscles, and volume of red corpuscles by the hematocrit, have given no indication of increased concentration of the blood. The sharp loss of body weight, amounting in some cases to as much

TABLE 1

*Showing the effect of the total loss of gastric juice on the blood chemistry (dog 1A)*

| DAYS | REMARKS | GASTRIC JUICE | | | | BLOOD CHEMISTRY | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | Volume | Free HCl | Total HCl | Per cent Cl | Cl | $CO_2$ | pH | Urea N | NPN |
| | | *cc.* | | | | *mgm.* | *cc.* | | *mgm.* | *mgm.* |
| 1 | Normal control period | | | | | 298 | 41 | 7.30 | 13 | 27 |
| 2 | Operation: Production of isolated stomach | | | | | 261 | 55 | | 14 | 27 |
| 3 | Ringer's solution 900 cc. | 600 | 0.40 | 0.43 | 0.44 | 210 | 53 | | 14 | 33 |
| 7 | Ringer's 1870 cc. | 1365 | 0.44 | 0.47 | 0.56 | 261 | 48 | | 13 | 24 |
| 8 | Ringer's 2000 cc. | 440 | 0.37 | 0.40 | 0.53 | 280 | 63 | 7.31 | 16 | 27 |
| 9 | No Ringer's | 190 | 0.23 | 0.55 | 0.58 | 208 | 75 | | 13 | 34 |
| 11 | No Ringer's | 242 | 0.22 | 0.29 | 0.47 | 171 | 76 | | 27 | 53 |
| 14 | 2000 cc. Ringer's daily for 3 days | 315 | 0.43 | 0.46 | 0.52 | 262 | 68 | | 13 | 32 |
| 25 | 2000 cc. Ringer's daily for 6 days | 432 | 0.44 | 0.47 | 0.49 | 268 | 43 | | 19 | 38 |
| 26 | No Ringer's | 368 | 0.41 | 0.45 | 0.49 | 220 | 93 | | 16 | 35 |
| 27 | No Ringer's | 313 | 0.35 | 0.38 | 0.49 | 151 | 108 | | 37 | 60 |
| 28 | No Ringer's; severe depression | 120 | 0.31 | 0.36 | 0.45 | 108 | 104 | 7.70 | 60 | 88 |
| 29 | 600 cc. 5 per cent NaCl by stomach tube | 238 | 0.36 | 0.43 | 0.48 | 145 | 120 | | 46 | 81 |
| 36 | 2000 cc. Ringer's daily for 7 days | 660 | 0.48 | 0.51 | 0.55 | 279 | 60 | | 27 | 45 |
| 47 | 2000 cc. Ringer's daily for 11 days | 815 | 0.48 | 0.52 | 0.57 | 266 | 70 | 7.63 | 20 | 39 |
| 48 | No Ringer's | 415 | 0.47 | 0.50 | 0.52 | 208 | 97 | 7.57 | 29 | 52 |
| 49 | No Ringer's | 260 | 0.40 | 0.45 | 0.49 | 183 | 108 | | 49 | 80 |
| 50 | No Ringer's | 122 | 0.33 | 0.46 | 0.50 | 177 | 114 | 7.95 | — | 90 |
| 51 | No Ringer's; severe depression | 250 | 0.35 | 0.42 | 0.53 | 110 | 120 | 7.73 | 65 | 80 |

as a kilo in 24 hours, must therefore be largely due to the removal of water from the tissues. The increase in non-protein and urea nitrogen occurs simultaneously with the urinary suppression and is probably to be accounted for on the basis of retention rather than increased breakdown of body protein. The urinary suppression in turn is doubtless the result of the tissue dehydration and possibly more specifically due to the hypochloremia. The data in table 3 indicate that even a partial loss of gastric juice may induce marked changes in the blood chemistry. In this animal a Pavlov accessory stomach pouch was made with the customary technique except that a very large proportion, perhaps two-thirds of the entire stomach, was included in the pouch.

3. *The symptoms may be relieved, the blood chemistry returned toward the normal and life prolonged, by the intravenous injection of 2,000 to 3,000 cc. of Ringer's solution per 24 hours.* The response to the injection was usually immediate. The depression and muscular weakness were promptly relieved and the appetite stimulated. Food that had remained unnoticed in the cage was promptly devoured. A striking effect, and one which will be referred to again later, was the production of thirst. Immediately after the intravenous injection of a liter of Ringer's solution the animal promptly sought the water jar and drank at least an equal amount of water that had been untouched for many hours. The effect of the injections on the blood chemistry is illustrated in the tables. In the case of dog 1-A (table 1) Ringer's solution was given by intravenous injection for the first 6 days after the production of the isolated stomach. Two thousand cubic centimeters per 24 hours sufficed to prevent any change in the blood chemistry. On the withdrawal of the Ringer's solution for 3 days the chlorides decreased from 280 to 171 mgm., the $CO_2$ capacity increased from 63 to 76 cc., the urea nitrogen increased from 16 to 27 mgm., and the non-protein nitrogen from 27 to 53 mgm. The administration of Ringer's solution (2000 cc. per day for 3 days) then restored the chlorides to 262 mgm., the $CO_2$ to 68 cc., the urea nitrogen to 13 mgm., and the non-protein N to 32 mgm. Numerous examples of this effect of Ringer's solution, in preventing the development of symptoms and blood chemistry changes from the loss of gastric juice and in correcting them after their occurrence, have been obtained during the course of the experiments. However, it should be noted that the response to the injections became steadily less complete, the animals continued to lose weight and strength, and finally even large injections failed to entirely restore the altered blood chemistry. All of the animals finally died, the survival after the operations being respectively 21, 30, 43 and 76 days. It is very probable that a much longer survival period could have been obtained had the daily injections of Ringer's solution not been occasionally interrupted. During these withdrawal periods the animals became so depressed that it is likely some irreversible

damage was done. It is very probable also that the digestive disturbance due to the loss of gastric function played an important part in the gradually increasing emaciation.

4. *The isolated stomach, prepared by the method described above with intact blood and vagal supply, may secrete in 24 hours from 1000 to 2600 cc. of gastric juice.* This secretion has repeatedly shown a chloride concentration of 0.50 to 0.55 per cent, free HCl 0.45 to 0.50 per cent, total HCl 0.50 to 0.55 per cent, and pepsin 100 to 150 units as determined by the Mett method. It is thus normal pure gastric juice. It is surprising in view of the fact that the stimuli for secretion here are entirely extra-gastric that such large quantities of juice have been obtained. No food whatever enters the stomach and the gastric juice itself does not remain in contact with the mucosa for any appreciable length of time. The importance of the vagi is suggested by the fact that in a similar preparation but with the vagi cut Ivy (1929) obtained less than half this volume of juice. However, it is probable that it is the presence of food substances in the duodenum and jejunum which constitutes the more important stimulus for gastric secretion in this preparation. The work of Luckhardt, F. C. Koch, and Ivy and his associates indicates that this stimulation is brought about by a hormone mechanism.

When these large volumes of gastric secretion pass directly to the exterior instead of into the lower intestine, it is clear that the body loses very considerable amounts of water and inorganic salts (chiefly chloride). Thus on one day animal 477-A (table 2) secreted 980 cc. of gastric juice with a chloride concentration of 0.457 per cent. The blood volume was 1490 cc. as determined by the dye method and the blood chloride 267 mgm. per 100 cc. Accordingly 4.522 grams of chloride ion were excreted in 24 hours when the total chloride content of the blood was 3.978 grams. By similar calculation animal 682-A secreting 2650 cc. of gastric juice lost 13.39 grams of chloride in 24 hours when the total chloride in the circulating blood was only 3.072 grams. In both of these instances the animal had been receiving chloride by intravenous injection in the Ringer's solution and consequently much of the chloride lost may have come from this source. However, on the 14th day animal 477-A had received no Ringer's solution for 48 hours. Nevertheless 580 cc. of gastric juice with a chloride concentration of 0.50 per cent were secreted during the day, making a total of 2.90 grams of chloride ion lost to the body. Before the period of collection the blood contained 3.63 grams of chloride and at the close of the period 3.045 grams. Thus of the total 2.90 grams of chloride lost to the body only 0.585 gram or less than one-fourth came from the blood while 2.315 grams or more than three-fourths of the total was removed from the tissues. During these 24 hours the animal lost 400 grams in weight. This probably also represented chiefly a removal of water from the tissues since deter-

TABLE 2

*Showing the effect of the total loss of gastric juice on the blood chemistry (dog 477A)*

| DAYS | REMARKS | GASTRIC JUICE | | | | BLOOD CHEMISTRY | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Volume | Free HCl | Total HCl | Per cent Cl | Cl | $CO_2$ | pH | Urea | NPN | RBC | Hb | Hemat. |
| | | *cc.* | | | | *mgm.* | *cc.* | | *mgm.* | *mgm.* | *mill.* | *grams* | *per cent RBC* |
| 1 | Normal control period | | | | | 266 | 63 | 7.31 | 21 | 41 | 6.7 | 15.7 | 52 |
| 2 | Normal control period | | | | | 249 | 56 | 7.35 | 17 | 36 | 6.7 | 15.7 | 52 |
| 3 | Operation: isolated stomach | | | | | | | | | | | | |
| 4 | 2000 cc. Ringer's | | | | | 268 | 51 | | 21 | 40 | | | |
| 5 | 2000 cc. Ringer's | 140 | 0.25 | 0.30 | 0.45 | 225 | | 7.30 | 14 | 27 | | | |
| 6 | 2000 cc. Ringer's | 400 | 0.16 | 0.18 | 0.19 | 268 | 71 | 7.43 | 14 | 26 | 5.5 | 14.0 | 52 |
| 7 | 2000 cc. Ringer's | 740 | 0.36 | 0.40 | 0.43 | 245 | 66 | 7.50 | 14 | 25 | | | |
| 8 | 2000 cc. Ringer's | 400 | 0.31 | 0.34 | 0.41 | 269 | 63 | 7.49 | 13 | 24 | | | |
| 9 | 2000 cc. Ringer's | 760 | 0.39 | 0.42 | 0.45 | 269 | 68 | 7.55 | 14 | 25 | | | |
| 10 | 2000 cc. Ringer's | 980 | 0.38 | 0.42 | 0.46 | 267 | 45 | 7.45 | 15 | 27 | | | |
| 11 | 2000 cc. Ringer's | 800 | 0.35 | 0.38 | 0.46 | 267 | 64 | 7.49 | 16 | 29 | | | |
| 13 | No Ringer's | 510 | 0.35 | 0.37 | 0.44 | 267 | 58 | 7.51 | 14 | 30 | 5.5 | 14.0 | 42 |
| 14 | No Ringer's | 580 | 0.31 | 0.34 | 0.50 | 242 | 64 | 7.44 | 17 | 35 | | | |
| 15 | No Ringer's | 445 | 0.22 | 0.25 | 0.46 | 203 | 91 | 7.62 | 25 | 44 | | | |
| 16 | No Ringer's | 340 | 0.16 | 0.21 | 0.43 | 186 | 76 | 7.49 | 21 | 48 | | | |
| 17 | No Ringer's | 275 | 0.19 | 0.25 | 0.39 | 173 | 100 | 7.67 | 22 | 41 | | | |
| 18 | No Ringer's | 228 | 0.15 | 0.21 | 0.39 | 160 | 102 | 7.61 | 30 | 52 | 5.9 | 15.8 | 43 |
| 20 | No Ringer's | 170 | 0.18 | 0.25 | 0.32 | 135 | 112 | 7.59 | 42 | 60 | | | |
| 25 | Died in depression; no evidence of tetany; weight loss from 21.4 kgm. to 16.0 kgm.; Temp. normal throughout | | | | | | | | | | | | |

minations showed no decrease in total blood volume and no alteration in the hematocrit, specific gravity, hemoglobin, or red cell counts. It is remarkable that the animal made no attempt to compensate for this water loss by drinking, although he had free access to both food and water. This point has been repeatedly observed in other dogs. If, however, under these conditions, 1000 cc. of Ringer's solution be injected intravenously, he promptly drinks a large amount of water. It would thus appear that thirst is related not so much to actual dehydration as to a relative disturbance in the balance between the total inorganic salt and water content of the body.

5. *The gastric glands can continue to secrete gastric juice of high free and total acidity even when the blood chloride has been reduced to less than a third of its normal concentration and an extreme alkalosis exists.* Thus on one day gastric juice with a free HCl of 0.31 per cent and a total chloride of 0.45 per cent was obtained from animal 1-A when the blood chloride was 108 mgm. per 100 cc., the plasma $CO_2$ 104 cc., and the pH 7.70. There is apparently no threshold below which the gastric glands cannot remove chloride ion from the blood. This finding may be reconciled since under normal conditions opportunity is afforded for the reabsorption of these gastric juice constituents in the lower intestine. On the other hand it makes possible the steady removal of the most important acid ion from the blood and tissues whenever conditions are such that gastric juice cannot reach the absorbing intestine. It is thus of very great significance in high intestinal obstruction, acute dilatation of the stomach, gastric and high intestinal fistulae, and profuse persistent vomiting. It is a striking fact that the gastric mucosa can bring about the death of the individual through the continued withdrawal from the blood of the constituents of gastric juice. Successful replacement therapy with sodium chloride indicates that it is the loss of these ions that is especially important.

6. While it is true that the secretion of gastric juice continues in spite of the marked chemical alterations in the blood plasma, the volume of secretion is lessened and its acidity reduced. This is demonstrated repeatedly in the tables. The withholding of Ringer's solution permits an immediate change in the blood chemistry and shortly therafter a decrease in volume and acidity of the escaping gastric juice. The decrease in secretion occurs before the developing anorexia limits food taking. Later, however, this latter factor becomes prominent and is perhaps largely responsible for the decreased secretion. Under these conditions, lessened gastric secretion in the presence of marked hypochloremia and alkalosis, the intravenous injection of Ringer's solution causes a profound increase in both volume and acidity of the gastric juice. This may occur entirely without the stimulus of food. The mechanism of this stimulation is not entirely clear. Sutherland (1921) observed it in animals provided with a Pavlov pouch. Our

TABLE 3

*Showing the effect of partial loss of gastric juice (Pavlov pouch) on blood chemistry (dog 875A)*

| DAYS | REMARKS | GASTRIC JUICE | | | | BLOOD CHEMISTRY | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Volume | Free HCl | Total HCl | Per cent Cl | Cl | $CO_2$ | pH | Urea | NPN | RBC | Hb | Hemat. |
| | | *cc.* | | | | *mgm.* | *cc.* | | *mgm.* | *mgm.* | *mill.* | *gram* | *per cent RBC* |
| 1 | Normal control period | | | | | 244 | 53 | 7.40 | 13 | 32 | 6.4 | 16.4 | 41 |
| 2 | Operation; large Pavlov pouch | | | | | 295 | 41 | 7.23 | 14 | 31 | | | |
| 3 | 2000 cc. Ringer's | | | | | 274 | 50 | 7.43 | 13 | 28 | | | |
| 4 | No Ringer's | 830 | 0.33 | 0.42 | 0.49 | 255 | 80 | 7.67 | 15 | 33 | | | |
| 6 | No Ringer's | 725 | 0.39 | 0.45 | | 202 | 105 | 7.57 | 21 | 42 | | | |
| 7 | No Ringer's. Marked depression | 500 | 0.24 | 0.32 | 0.34 | 176 | 96 | 7.52 | 27 | 42 | 6.4 | 16.4 | 41 |
| 8 | 2000 cc. Ringer's | 1050 | 0.43 | 0.47 | 0.46 | 235 | 70 | 7.66 | 23 | 39 | | | |
| 9 | 2000 cc. Ringer's. Lively | 1100 | 0.43 | 0.46 | 0.49 | 300 | 49 | 7.67 | 22 | 31 | | | |
| 14 | 2000 cc. Ringer's daily for 5 days | 2800 | 0.49 | 0.53 | | 319 | 66 | 7.61 | 19 | 36 | | | |
| 18 | 2000 cc. Ringer's daily for 4 days | 1850 | 0.44 | 0.50 | | 261 | 38 | 7.64 | 18 | 38 | | | |
| 22 | 2000 cc. Ringer's daily for 4 days | 1280 | 0.45 | 0.46 | 0.48 | 255 | 60 | 7.40 | 16 | 34 | | | |
| 26 | 2000 cc. Ringer's daily for 4 days | 1740 | 0.43 | 0.48 | 0.48 | 209 | 81 | 7.37 | 14 | 36 | 6.5 | 15.0 | 41 |
| 28 | No Ringer's for 2 days | 565 | 0.36 | 0.42 | 0.48 | 138 | 88 | 7.44 | 22 | 43 | | | |
| 29 | No Ringer's; depressed | 870 | 0.38 | 0.44 | 0.42 | 116 | 109 | 7.54 | 42 | 67 | | | |
| 30 | 1000 cc. Ringer's | 960 | 0.21 | 0.25 | 0.33 | 135 | 85 | 7.49 | 23 | 46 | 5.0 | 13.5 | 40 |

observations suggest that the restoration of the normal chemical state of the plasma is an important factor since this should decrease the amount of work necessary for the concentration of chloride ion in its passage from the plasma through the gastric gland cell to the stomach lumen. It is at all events clear that gastric secretion is profoundly affected by alterations in the chemistry of the blood and this factor should be taken into consideration in studies of gastric secretory function where partial or complete loss of gastric juice (such as occurs in gastric fistulae, the Pavlov pouch, and preparations similar to ours) takes place.

Clinical application has been made of the findings described above, the details of which are to be published elsewhere. It has become apparent that failure of reabsorption of gastric juice in the lower intestine is the chief cause of death in high intestinal and pyloric obstruction, acute dilatation of the stomach, paralytic ileus in general peritonitis, gastro-colonic fistula, duodenal and gastric fistulae, and profuse persistent vomiting from other causes. A number of such cases have been successfully treated by simple replacement therapy with intravenous physiological salt solutions.

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# THE ABILITY OF THE COMMON MACKEREL AND CERTAIN OTHER MARINE FISHES TO REMOVE DISSOLVED OXYGEN FROM SEA WATER

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The maintenance of protoplasmic activity is conditioned by a supply of oxygen. Many of the physiological processes are oxidations which can not continue without an abundant supply of oxygen. The quantity of oxygen provided to organisms by their environment varies. The quantity of oxygen present in the environment is not the only concern of the organism. The *tension* or *partial pressure* of that gas may also limit its physiological activities. The sea with its small quantity of oxygen per unit volume maintains generally a high oxygen tension. The surface layers of the sea are nearly in equilibrium with the air, which is about 159 mm. of mercury. Low oxygen tensions, however, have been reported in deep water which lies stagnant in hollows in the sea bottom (Harvey, 1928). On the other hand, estuaries close in shore may have high oxygen tension due to the photosynthesis of a rich flora. Intertidal pools may have either a high or a low oxygen tension, depending upon the quantity and kind of life present.

Animal life is found in all of the above mentioned conditions and is affected in various manners by differences in oxygen tension. It appears that some marine fishes have an oxygen consumption independent of the tension within a wide range, while others have the oxygen consumption limited by the tension at all points (Hall, 1929a). The scup, an active fish, seems to be of the former type and the sluggish toadfish of the latter.

It occurred to the writer that it might be of interest to find out the ability of several species of marine fishes to remove dissolved oxygen from sea water. In other words, to determine at what partial pressure fishes fail to remove oxygen from their surroundings.

METHODS. The apparatus and methods employed were similar to those of a previous investigation (Hall, 1929a). Individual fish were placed in containers of about 40 times that of their volume. The water in which the fish were submerged was saturated with air at 20°C., and 760 mm. Hg. The containers were then closed. Samples were drawn just as soon as all respiratory movements ceased. The dissolved oxygen was determined

by the Winkler method. The temperature was maintained constant throughout each experiment.

RESULTS. The results obtained on 11 species of teleost fishes show that a considerable variation occurs in their ability to remove dissolved oxygen from their surroundings. There appears to be one group of fishes, scup, cunner, rudderfish, and butterfish that cease respiration at oxygen tensions between 11 and 16 mm. Hg pressure. Another group, such as the puffer and sea bass, remove the oxygen to a much lower tension, 3 to 5 mm. The tautog and toadfish remove practically all of the oxygen. Not enough oxygen was left in water in which toadfish were submerged to even detect

TABLE 1

*Showing the lowest oxygen tension at which fishes are capable of removing dissolved oxygen*

The iron and sugar concentration values are given for comparison. (Scientific names are omitted for sake of brevity. They have been given in a previous paper (Hall, 1929b).)

| SPECIES | NUMBER OF INDIVIDUALS | OXYGEN TENSION | IRON PER 100 CC. OF BLOOD | SUGAR PER 100 CC. OF BLOOD |
|---|---|---|---|---|
| | | *mm. Hg.* | *mgm.* | *mgm.* |
| Mackerel | 8 | 70 | 43.8 | 53.4 |
| Rudder-fish | 10 | 15.1 | 21.7 | 67.7 |
| Cunner | 8 | 14.8 | 27.7 | — |
| Scup | 10 | 13.1 | 25.3 | 60.1 |
| Butter fish | 12 | 11.8 | 27.4 | 68.9 |
| Sea Robin | 9 | 8.5 | 23.7 | 38.8 |
| Fundulus | 6 | 8.1 | — | — |
| Puffer | 10 | 4.7 | 21.5 | 23.6 |
| Sea Bass | 8 | 3.2 | — | — |
| Tautog | 8 | 1.6 | 18.0 | 19.6 |
| Toadfish | 10 | 0.0 | 13.5 | 9.0 |

its presence by the Winkler method. Summarized results are shown in table 1, which represent the average for all individuals of each species. Only slight individual deviation from the average for the species obtained.

The striking difference in the ability of the common mackerel, as compared with other fishes, to remove dissolved oxygen leads to further study. Repeated experiments showed that mackerel cannot live long in a closed jar and are able to remove only a small proportion of the dissolved oxygen. Obviously they have some peculiar mechanism of respiration. The first clue to an explanation was disclosed when an attempt was made to anesthetize mackerel. The quantity of urethane necessary to just completely anesthetize scup, preventing locomotion without influencing respiratory movements, had previously been determined. When this same amount of

urethane was given mackerel they quickly succumbed. It was found that any small quantity required to prevent locomotion would kill the mackerel. Death appeared to be due to asphyxia, even though an abundant supply of oxygen was available. Consequently, it seemed probable that in some manner intensity of locomotor activity might be concerned in the ability to remove dissolved oxygen.

As a test of this possibility mackerel were subjected to three conditions. Some were kept in a floating cage 14 feet long, thus permitting them to swim freely and rapidly without obstruction. A second group were prevented from rapid locomotion by Erlenmeyer flasks attached to their tails. They were, however, permitted to swim slowly in any direction. Individuals of the last group were placed in screen cages slightly longer than their bodies. Thus no forward locomotion was possible. They were kept in circulating sea water saturated with air. After an interval of fifteen minutes fishes from each group were bled from the caudal artery into

TABLE 2

*The influence of locomotion on the oxygen saturation of the blood of the common mackerel*

| LOCOMOTION | OXYGEN SATURATION OF BLOOD | DISSOLVED OXYGEN IN SURROUNDING WATER |
|---|---|---|
| | *per cent* | *cc. per liter* |
| Forward rapid unobstructed | 85 | 5 0 |
| Forward retarded with Erlenmeyer flask attached to tail | 46 | 5 0 |
| Stationary obstructed by screen cage | 11 | 5 0 |

Erlenmeyer flasks. The blood was collected under oil without exposure to air and prevented from clotting by the addition of 2 mgm. of lithium oxalate per cubic centimeter of blood. Each sample of blood thus procured was divided into two parts. One part was used to determine the actual oxygen content. The other part was equilibrated with air to determine the oxygen capacity. The Van Slyke manometric gas analysis apparatus was used with the usual procedure as directed by Van Slyke and Neill, 1924. Hence, knowing the oxygen capacity and the actual amount of oxygen carried by the blood, the percentage of saturation of the blood of the fishes which were subjected to each of these conditions could be ascertained. The percentage of saturation of blood with oxygen is therefore taken as the index of the ability of mackerel to remove dissolved oxygen under various circumstances. The results are summarized in table 2.

Discussion. The results of this investigation indicate that marine fishes vary with the species in their ability to remove dissolved oxygen. Some are able to remove the oxygen to a very low partial pressure, others succumb from asphyxiation at a much higher partial pressure. Those spe-

cies capable of lowering the oxygen tension to the greatest extent are sluggish forms. The toadfish was apparently able to remove all of the oxygen before death occurred. The more active forms such as the scup, butterfish, and rudderfish died at a partial pressure of 12 to 16 mm. Hg. The scup has been shown to have a relatively constant oxygen consumption at oxygen tension above 40 mm. (Hall, 1929a). The oxygen consumption of the toadfish is almost directly proportional to the oxygen tension at all points. It is perhaps a significant observation that species capable of removing oxygen at the lowest tension have the lowest hemoglobin concentration, while the more active species incapable of removing oxygen at low tensions have the highest hemoglobin concentration. Further studies on the oxygen dissociation curves of these species would be of interest.

The aspect presented by the common mackerel is considerably different from that of fishes discussed above. The results of this investigation indicate that in some manner the respiratory mechanism is correlated with that of locomotion. Perhaps one should first consider the habits of this species. It is a pelagic animal feeding mainly on plankton. It feeds by straining great quantities of water over its gills. Copepods and other animal organisms are collected by highly developed gill rakers. Mackerel swim in schools, rapidly moving from place to place in search of copepods, their principal food. They are migratory fishes. The extent of their migrations and the factors which influence their wanderings are not well known. The investigations conducted by Mr. O. E. Sette, U. S. Bureau of Fisheries, furnish the most extensive information on this subject.

The mechanism of respiration in fishes generally consists in rhythmical suction of water into the oral cavity and its subsequent expulsion through the gill clefts. During inspiration the mouth is opened and the oral cavity enlarged by the lateral expansion of its walls. When the oral cavity is closed the expiratory process begins. By the lateral contraction of the oral walls the water is driven through the gill-clefts and over the gill filaments. The branchial arches become separated allowing all of the gill filaments to come in contact with the circulating water. In this manner the blood is aerated.

Swimming rapidly through the water with open mouth enables mackerel to strain great quantities of water through their gill clefts. The primary function is to obtain food, but coincidently it appears that another advantage is gained, for when water is strained by the gill rakers it passes over the gill filaments, and, in consequence, the blood is aerated. Therefore no suction action is needed as long as forward locomotion is maintained. In fact it appears to be lost for this species. It seems from the evidence that there exists a correlation between the feeding habits of mackerel and their breathing mechanism. In feeding they must move rapidly, likewise, perforce, to aerate their blood they must move forward in a similar manner.

Possibly this correlated function of feeding and breathing by locomotion has some bearing upon the migration of mackerel. But at present there is not sufficient knowledge of the phenomena of migration to warrant speculation. These findings will explain the practical difficulty which has been experienced in keeping mackerel alive in aquaria. One can easily see the the futility of restricting the common mackerel to small aquaria even though the oxygen concentration may be high for with retarded forward locomotion such mackerel will become asphyxiated in the presence of an abundant oxygen supply. Elongated tanks partially overcome the difficulty.

## SUMMARY

1. The toadfish is able to remove all the measurable dissolved oxygen from water in which it is confined.

2. In general fishes remove dissolved oxygen to a partial pressure of 2 to 16 mm. Hg. Each species has its own limits. The sluggish ones are capable of removing oxygen to low tensions, the active forms are less capable.

3. The common mackerel is not able to respire sufficiently unless it is moving rapidly forward. Ability to respire in a stationary position has been practically lost.

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# THE CARBON DIOXIDE CAPACITY OF THE BODY AND THE RATE AT WHICH THE BODY COMES INTO EQUILIBRIUM WITH CHANGES IN THE ALVEOLAR CARBON DIOXIDE TENSION

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The present investigation was undertaken to determine the carbon dioxide capacity of the body and the rate at which the body comes into equilibrium with changes in the carbon dioxide tension of the alveolar air.

A cat was made to breathe a gas mixture containing about 11 per cent carbon dioxide for a sufficient length of time to insure the equilibrium of the entire body with the increased carbon dioxide tension of the alveolar air. Outdoor air was then substituted for the gas mixture, and the expired air collected periodically and analyzed for carbon dioxide and oxygen. During this desaturation period the respiratory quotient was higher than that normal to the cat, due to the elimination of the carbon dioxide retained, and gradually fell to a value which remained constant during the rest of the experiment, and which we assumed to represent the true ratio of oxygen consumption to carbon dioxide production during the experiment. The carbon dioxide that was retained by the body, and subsequently eliminated in response to the lowered alveolar carbon dioxide tension, could then be evaluated by subtracting the metabolic carbon dioxide from the total carbon dioxide. An estimation of the retained or excess carbon dioxide in a series of collections of expired air, when correlated with time, gave the rate at which equilibrium was reached during the desaturation process; and by adding the quantities thus determined for each collection, the total quantity of carbon dioxide retained by the body was derived.

METHOD. A cat was given an injection of 5 per cent barbital sodium solution, which was allowed to run slowly from a burette into the femoral vein. The dose varied from 0.30 to 0.35 gram per kilogram of body weight. A cannula was then inserted in the trachea and connected with respiratory valves.

A steel pressure cylinder was charged in the laboratory with carbon dioxide (10 to 12 per cent), oxygen (25 per cent) and nitrogen. This gas mixture was allowed to flow into a spirometer, the latter being connected

to the inspiratory valve. Ninety minutes were allowed for the tissues of the body to come into equilibrium with the increase in alveolar carbon dioxide tension.

At the termination of the saturation period the inspiratory valve was connected to a spirometer containing outdoor air, and the expiratory valve was connected to a three-way cock in such a manner that the expired air might be collected in either one of two spirometers, one having a capacity of 6 liters and the other of 25 liters. The smaller spirometer was used to collect the expired air during the interval required to read the volume and take a sample of the gas in the larger spirometer. The latter was then emptied and the gas collected in the small spirometer was shunted into the large spirometer, thus permitting a continuous collection with periodic

TABLE 1

*Protocol for experiment 33*

| COLLECTION | TIME | VOLUME | | $CO_2$ | $O_2$ | R.Q. |
|---|---|---|---|---|---|---|
| | | Observed | At standard conditions | | | |
| | *p.m.* | *cc.* | *cc.* | *per cent* | *per cent* | |
| 1 | 2:00-2:05 | 8,723 | 7,729 | 2.712 | 19.427 | 2.229 |
| 2 | 2:15 | 9,497 | 8,414 | 2.701 | 18.471 | 1.106 |
| 3 | 2:30 | 13,621 | 12,068 | 2.461 | 18.401 | 0.947 |
| 4 | 2:45 | 14,827 | 13,136 | 2.225 | 18.537 | 0.893 |
| 5 | 3:00 | 15,102 | 13,380 | 2.097 | 18.652 | 0.881 |
| 6 | 3:25 | 25,007 | 22,156 | 2.013 | 18.684 | 0.852 |
| 7 | 3:45 | 20,917 | 18,532 | 1.959 | 18.640 | 0.804 |
| 8 | 4:05 | 22,329 | 19,783 | 1.901 | 18.707 | 0.803 |
| 9 | 4:20 | 21,546 | 19,090 | 1.877 | 18.737 | 0.804 |
| 10 | 4:40 | 23,500 | 20,820 | 1.830 | 18.807 | 0.810 |

determinations. Observations upon the desaturation process were made over a period of three hours.

At the termination of the saturation process and just prior to collecting the expired air during the desaturation process, the tracheal cannula was closed off and the lungs held in the extreme expiratory phase for a few seconds while the respiratory valves and tubing were rinsed with room air to clear them of the inspired gas mixture. Though the residual air in the lungs still contained the inspired gas mixture, the volume was too small to have an appreciable effect upon our calculations for carbon dioxide retained by the body.

The protocol for a typical experiment is given in table 1. At 12:30 p.m., the cat started to breathe a gas mixture containing 12.11 per cent carbon dioxide and about 25 per cent oxygen, and continued until 2:00 when out-

door air was substituted. There was a gradual fall in the respiratory quotient until the seventh collection, and thereafter the respiratory quotient remained relatively constant for the remainder of the experiment, with an average value of 0.805. In this experiment the desaturation process was complete at 3:25, 85 minutes after the fall in the alveolar carbon dioxide tension.

If we assume that the respiratory quotient which appears immediately after desaturation was held during desaturation, then in the experiment quoted the metabolic carbon dioxide is 0.805 times the oxygen consumed, and total carbon dioxide minus metabolic carbon dioxide equals retained carbon dioxide. For example, during period 1, 207.3 cc. of carbon dioxide were excreted and 93.0 cc. of oxygen absorbed. The metabolic carbon dioxide was, therefore, $0.805 \times 93.0 = 74.4$ cc., and the retained carbon dioxide was $207.3 - 74.4 = 132.9$ cc. In the same manner the volume of retained carbon dioxide was estimated for each collection period, and the sum of the volumes indicates the total volume of carbon dioxide retained by the entire body.

It is important that the respiratory quotient should be both constant and accurately determined, since the estimation of the quantity of carbon dioxide formed in the body during the desaturation process depends upon this factor. We have found that a respiratory quotient taken too soon after the administration of an anesthetic tends to fluctuate. In our experiments, therefore, the sodium barbital was given five hours prior to the desaturation period, and was administered intravenously instead of intraperitoneally to hasten still further its absorption. The animals experimented upon received no food for a period of 20 hours or more prior to the determination of the respiratory quotient, in order that they should be in the post-absorptive state.

There are two sources of significant experimental error: 1, the analytical error in determining the carbon dioxide and oxygen of the expired air, and 2, the possibility that the respiratory quotient during desaturation may not be identical to that taken either before or after desaturation.

The analytical error for the determination of carbon dioxide was $\pm 0.015$ per cent, and for oxygen $\pm 0.020$ per cent. In the experiment cited (see table 1), the carbon dioxide retained by the body was derived from gas analyses made upon six samples. The additive effect of the maximum error in each analysis would result in an error of about 10 per cent in the estimation of the total carbon dioxide retained by the body.

The determination of the respiratory quotient during the hour that follows desaturation is the average of three or four collections taken at 15 or 20-minute intervals. The error involved in making this determination when the respiratory quotients are in close agreement is probably insignificant, and unless the agreement was within $\pm 0.01$ the experiment

was discarded. There remains, however, the possibility that the true respiratory quotient during desaturation may have been slightly different from a determination made subsequent to desaturation. Notwithstanding the constancy of a respiratory quotient over a period of three hours in an animal which is in the post-absorptive stage, we feel that in estimating our experimental error an allowance of $\pm 0.02$ should be made for a departure of the true respiratory quotient during desaturation from that taken after desaturation. In the experiment quoted as an example, a departure of 0.02 from the observed respiratory quotient would result in an error of about 12 per cent in the quantity of carbon dioxide retained by the body.

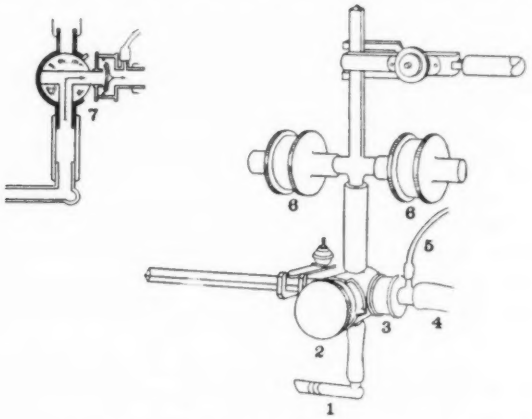


Fig. 1. Apparatus for collection of alveolar air and respiratory valves. *1*, tracheal cannula; *2*, three-way cock; *3*, valve to prevent intake of alveolar air back into lungs; *4*, collecting tube; *5*, sampling tube; *6*, respiratory valves; *7*, section of three-way cock.

The analytical error of 10 per cent, plus an error of 12 per cent in determining the correct respiratory quotient, results in a possible error of 22 per cent in estimating the quantity of carbon dioxide retained by the body.

In order that the carbon dioxide retained by the body may be correlated with the rise in the alveolar carbon dioxide tension, samples of alveolar air were taken at the end of the saturation period and again at the end of the desaturation period. The carbon dioxide retained by a unit weight of tissue per millimeter increase in alveolar carbon dioxide tension may then be estimated.

The apparatus for collecting alveolar air is shown in figure 1. It is connected at one end to the tracheal cannula, and at the other end to the respiratory valves. When the chest is at full inspiration, the three-way

cock *2* is turned so that the expired air passes through the valve on the side-arm into a rubber tube *4* measuring 75 cm. in length and 8 mm. bore. At the end of expiration the three-way cock is again turned back to restore normal breathing through the respiratory valves without interruption. It is impossible to withdraw air from the collecting tube back in the direction of the lungs owing to the presence of a valve *3* which is absolutely competent. The samples were drawn from the rubber tube into a glass collecting tube by mercury displacement. Calibrations were etched on the glass tube so that samples of any given volume might be collected. We found that the last 4 cc. of an expiration from cats of the size which we were using consisted of alveolar air. Therefore, in order to obtain sufficient air for the routine Haldane analysis, 4 cc. samples from five successive expirations were taken to make up a total volume of 20 cc.

We may look upon the expired air which passes into the rubber tube as a column of air of which the portion proximal to the lungs is alveolar air, which gradually becomes diluted with air from the dead spaces as the distal end of the tube is reached. The volume of alveolar air which enters the rubber tube was determined by taking samples of increasing volume. When the volume of the sample exceeds a given magnitude the carbon dioxide content begins to fall, which is evidence that dead-space air is beginning to dilute the alveolar air. The results of an experiment to determine the volume of alveolar air entering the rubber tube may be cited. Analyses were made of the carbon dioxide content of the last 2, 4, 6, and 8 cc. of expired air. These showed an alveolar carbon dioxide tension of 35.9, 35.0, 35.0, and 34.8 mm. of mercury, respectively. Thirty minutes later the same procedure was repeated on the same animal, showing an alveolar carbon dioxide tension of 35.3, 35.6, 35.4, and 34.4. It will be observed that samples containing the last 2, 4, and 6 cc. of an expiration had a carbon dioxide content which was relatively constant, showing that the last 6 cc. of the column of air in the rubber tube was of alveolar origin. The 8 cc. samples, however, were slightly lower in carbon dioxide content, indicating the onset of dilution with dead-space air.

It is important that the alveolar carbon dioxide tension should remain relatively constant during both the saturation and desaturation periods if a state of equilibrium is to be attained. That this is the case a typical experiment will serve as an illustration. Samples of alveolar air taken every ten minutes during a period of one hour, while the cat was breathing a gas mixture containing about 11 per cent carbon dioxide, gave the following carbon dioxide tensions in millimeters of mercury: 88.6, 88.8, 90.1, 90.9, 90.8, and 90.6. It is apparent that the respiratory center maintains its delicate setting notwithstanding the abnormal conditions imposed upon it.

The metabolic rate of the cat throughout the experiment was found to

be almost identical to that of an intact, unanesthetized animal. The average rate of oxygen consumption per minute per kilogram of body weight, for six intact cats in a metabolism chamber, was 7.3 cc.; for five cats under sodium barbital anesthesia, 7.9 cc.; and for five anesthetized cats following the inhalation of a 10.7 per cent carbon dioxide gas mixture was 7.8 cc. It is clear, therefore, that neither the anesthesia nor the carbon dioxide inspired had any appreciable effect upon the basal metabolism of our animals throughout the experiment.

TABLE 2

*The carbon dioxide capacity of the whole body and of the tissue fluids exclusive of blood*

| EXPERIMENT | WEIGHT OF CAT | ALVEOLAR $CO_2$ TENSION | | | $CO_2$ | | | BLOOD $CO_2$ / TOTAL $CO_2$ |
|---|---|---|---|---|---|---|---|---|
| | | On gas mixture | On air | Difference | Retained by body (total) | Per kilo body weight per millimeter tension difference | Per kilo tissue fluid per millimeter tension difference | |
| (1) | (2) | (3) | (4) | (5) | (6) | (7) | (8) | (9) |
| | *kgm.* | *mm. Hg* | *mm. Hg* | *mm. Hg* | *cc.* | *cc.* | *cc.* | *per cent* |
| 28 | 2.7 | 85.4 | 31.4 | 54.0 | 186 | 1.28 | 1.70 | 13.8 |
| 38 | 3.6 | 84.5 | 35.3 | 49.2 | 237.2 | 1.34 | 1.80 | 13.1 |
| 37 | 3.3 | 87.5 | 31.9 | 55.6 | 255.8 | 1.39 | 1.89 | 12.6 |
| 29 | 2.7 | 87.4 | 35.4 | 52.0 | 202.8 | 1.44 | 1.97 | 12.2 |
| 33 | 3.5 | 90.8 | 30.1 | 60.7 | 313.9 | 1.48 | 2.02 | 11.9 |
| 39 | 3.7 | 85.8 | 30.2 | 55.6 | 368.9 | 1.79 | 2.51 | 9.8 |
| 36 | 3.0 | 92.9 | 35.4 | 57.5 | 314.2 | 1.82 | 2.56 | 9.6 |
| 31 | 2.8 | 86.7 | 29.9 | 56.8 | 356.2 | 2.24 | 3.20 | 7.9 |
| 40 | 2.5 | 86.8 | 27.7 | 59.1 | 347.1 | 2.35 | 3.37 | 7.5 |
| 30 | 2.7 | 86.4 | 44.5 | 41.9 | 301.9 | 2.67 | 3.86 | 6.6 |
| Average..... | | 87.4 | 33.2 | 54.2 | | 1.78 | 2.49 | 10.5 |

Experiments arranged in order of increasing $CO_2$ combining capacity of tissue fluids.

EXPERIMENTAL RESULTS. The capacity of the body to absorb carbon dioxide is shown in column 7 of table 2, and was estimated on the basis of the carbon dioxide which was retained by 1 kgm. of body weight following a rise of 1 mm. of mercury in the alveolar carbon dioxide tension, or

$$\frac{\text{Total cc. of } CO_2 \text{ retained}}{\text{Kgm. of body weight} \times \text{mm. tension difference}} = \begin{cases}\text{Cc. of } CO_2 \text{ per kgm. of body wt.} \\ \text{per mm. tension difference}\end{cases}$$

In column 9, the ratio which the carbon dioxide retained by the blood bears to the carbon dioxide retained by the entire body has been estimated.

It has been assumed that the blood volume of a cat is about 5.5 per cent of its body weight. The volume of carbon dioxide absorbed by the blood

was derived from the carbon dioxide dissociation curve. Carbon dioxide dissociation curves for cats' blood were constructed from the blood of nine animals. That portion of the curve which lies between 30 and 90 mm. of carbon dioxide tension shows the volume of carbon dioxide taken up by the blood following a rise in the alveolar carbon dioxide tension which falls within the range of our experiments. It was found that 1 kilo of blood would absorb an average of 192 cc. of carbon dioxide when the tension was increased from 30 mm. to 90 mm. of mercury pressure, or 3.2 cc. per millimeter increase. Therefore, 0.055 × body weight in kgm. × 3.2 × mm. of $CO_2$ tension difference = cc. of $CO_2$ retained by the blood. The only

TABLE 3

*Rate of desaturation*

| EXPERIMENT | PER CENT DESATURATION | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | 30 | 40 | 50 | 60 | 70 | 80 | 90 | 100 |
| | *minutes* | *minutes* | *minutes* | *minutes* | *minutes* | *minutes* | *minutes* | *minutes* |
| 28 | | | 2.5 | 4.0 | 7.7 | 15.6 | 27.0 | 45.0 |
| 29 | 3.5 | 7.0 | 11.5 | 17.0 | 24.0 | 34.5 | 47.0 | 70.0 |
| 38 | | | 5.5 | 12.0 | 19.6 | 28.6 | 41.0 | 75.0 |
| 33 | | | 8.0 | 13.5 | 22.0 | 36.0 | 55.0 | 85.0 |
| 30 | 5.0 | 12.0 | 20.5 | 29.3 | 39.8 | 52.0 | 66.0 | 90.5 |
| 31 | 4.0 | 9.7 | 17.0 | 28.0 | 41.5 | 58.5 | 80.0 | 107.5 |
| 37 | 9.0 | 15.5 | 23.0 | 32.0 | 43.0 | 58.5 | 83.0 | 125.0 |
| 36 | 4.5 | 8.0 | 13.0 | 21.0 | 32.4 | 51.0 | 89.0 | 140.0 |
| 39 | 5.0 | 11.8 | 20.0 | 31.0 | 46.0 | 69.0 | 99.0 | 140.0 |
| 40 | 6.5 | 11.8 | 18.8 | 28.7 | 41.5 | 58.0 | 79.5 | 140.0 |
| Average | | | 14.0 | 21.6 | 31.7 | 46.2 | 66.7 | 101.8 |

Time in minutes to attain a given per cent of desaturation. Experiments arranged in order of increasing desaturation rate.

significant error involved in this calculation is to be found in the estimation of blood volume based upon body weight.

Since the carbon dioxide retained by the body is held in solution by the water of the body, composed of blood and tissue fluid, it is of interest to compare the buffering capacity of these two fluids. We have seen that 1 kgm. of cats' blood can absorb 3.2 cc. of carbon dioxide per millimeter of carbon dioxide tension difference. From our data we may now estimate how much carbon dioxide 1 kgm. of tissue fluid will absorb under similar conditions.

We may assume that the body is 70 per cent water by weight. Therefore, 1000 grams of mixed tissue will contain 700 grams of water, of which 55 grams is blood and 645 grams is tissue fluid. Therefore, the tissue fluid

is 0.645 times the body weight, and the cubic centimeters of carbon dioxide retained by 1 kgm. of tissue fluid per millimeter of carbon dioxide tension difference will be:

$$\frac{\text{Cc. } CO_2 \text{ retained by tissues (exclusive of blood)}}{\text{Kilos tissue fluid} \times \text{mm. tension difference}}$$

The carbon dioxide combining capacity of the tissue fluid, estimated in this manner, is given in the last column of table 2.

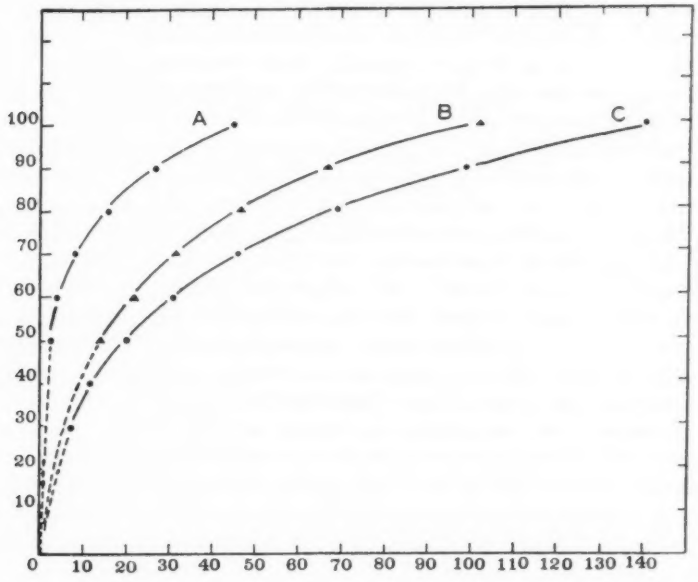


Fig. 2. Rate of desaturation. *Curve A*, experiment 28, representing minimum equilibrium time; *curve B*, average for all experiments as shown in table 3; *curve C*, experiment 39, representing maximum equilibrium time.

During the desaturation period the expired air was collected, as explained above, in about ten separate collections, from each of which a sample was taken for analysis. The per cent desaturation, which has been attained at any given time after desaturation commences, is the ratio which the retained carbon dioxide eliminated during that time bears to the total carbon dioxide retained. Curves for the percentage of desaturation plotted against time were constructed for each experiment, and the data taken from these curves are given in table 3. In figure 2, curves A and C are taken from experiments 28 and 39 and represent the minimum and maximum time

required for desaturation; curve B represents the average desaturation time for all the experiments.

Discussion. The experiments which we have described are similar in principle to experiments carried out by Shaw (1926) on cats, by Brocklehurst and Henderson (1927) on man, and by Adolph, Nance and Shiling (1928) on man. These investigators have used either one or both of two procedures, which may be termed the accumulative method and the eliminative method. By the accumulative method the carbon dioxide tension of the body is raised above the normal level by the inhalation of a gas mixture rich in carbon dioxide, and the volume of carbon dioxide retained by the body is measured. By the eliminative method the carbon dioxide tension of the body is depressed below the normal level by forced breathing, and the carbon dioxide which appears in the expired air in excess of that produced by current metabolism is measured.

Whichever method is used, it is obviously a matter of fundamental importance to maintain a constant alveolar carbon dioxide tension—above the normal level if the accumulative method is used, and below the normal level if the eliminative method is used—for a period of time sufficient to establish equilibrium of all the tissues of the body with the altered carbon dioxide tension of the blood. The difficulty of carrying out such experiments upon human subjects becomes apparent when we consider the extreme discomfort of maintaining a significant increase or diminution in the alveolar carbon dioxide tension for a sufficient length of time to insure the establishment of equilibrium throughout the body.

In a series of five experiments on human subjects, Adolph, Nance and Shiling (1928) induced a rise in the alveolar carbon dioxide tension ranging from 5 mm. to 19 mm. of mercury, which was sustained for a period of time ranging from 17 to 34 minutes. The variation in their results, computed on the basis of cubic centimeters retained by the body per millimeter change in alveolar carbon dioxide tension, is of the order of 1:17. Such wide variations are undoubtedly due in part to a failure to establish equilibrium between the carbon dioxide tension of the body and the alveolar air.

In the experiments of Brocklehurst and Henderson (1927), a man rebreathed the air in a bag containing 5 per cent or more of carbon dioxide for $2\frac{1}{2}$ minutes. Since the carbon dioxide tension in the bag and alveoli was steadily rising at a rate of 6 mm. of mercury or more per minute, a state of equilibrium was never reached. The time factor was ignored on the assumption that the carbon dioxide tension in the tissues throughout the body was increased at the same rate as the tension of carbon dioxide in the rebreathing bag. Brocklehurst and Henderson estimated that the capacity of the tissues, exclusive of the blood, to retain carbon dioxide between 40 to 50 mm. tension was 0.022 volume per cent for a pressure

change of 1 mm. of mercury. Under identical conditions human blood will retain 0.45 volume per cent and water 0.079 volume per cent. We must, therefore, conclude from their experiments that the tissues are only about one-twentieth as effective as blood as a buffering agent, and somewhat less than one-third as effective as water.

In table 2, column 6, it will be seen that the average volume of carbon dioxide retained per kilo of body weight per millimeter of alveolar carbon dioxide tension difference was 1.78 cc. In comparing the carbon dioxide combining capacity of the body with that of the blood, we find that the former is about 40 to 83 per cent as effective as the latter as a buffering agent.

Fenn (1928) has constructed the dissociation curve for the carbon dioxide combining capacity of nerve and muscle taken from the frog. He used excised tissue which was equilibrated *in vitro* at various carbon dioxide tensions. His method has the advantage of being direct. He found that the muscle and nerve had a carbon dioxide combining capacity from 33 to 50 per cent as great as the blood. Though the range of his values is somewhat lower than our own, we must make allowance not only for the differences that may be inherent in animals so diversified as the frog and the cat, but we must also bear in mind that the body may contain tissues having an alkali reserve much greater than nerve or muscle, which would result in raising the average for the body as a whole.

The ratio which the carbon dioxide retained by the blood bears to the carbon dioxide retained by the whole body is shown in the last column of table 2. Here we find that the blood is responsible for buffering about 10.5 per cent of the total acid retained by the body. This is in very close agreement with the experiments of Shaw (1926), using the accumulative method, who found that the blood buffered about 11.8 per cent of the total carbon dioxide retained.

When we estimate the carbon dioxide combining capacity of the tissue fluids, a simple calculation based on the fact that the carbon dioxide retained by the tissues (exclusive of blood) is held by the water of the body, we find that the average combining capacity of the tissue fluids was 2.49 cc. of carbon dioxide per kilo of fluid per millimeter of carbon dioxide tension difference, or 78 per cent of the blood capacity. In three of our experiments the capacity of the tissue fluids was equal to or greater than that of the blood.

These results, however, are not inconsistent with our knowledge of the carbon dioxide content of lymph and cerebrospinal fluid, which tends to approximate the carbon dioxide content of the plasma. Collip and Backus (1920) and Hamilton (1925) found that the carbon dioxide content of the spinal fluid was about 85 per cent as great as that of the serum when these were drawn simultaneously from human subjects. Collip and

Backus (1920) found that the carbon dioxide content of lymph taken from dogs was almost identical to that of the blood. These facts lead us to believe that the carbon dioxide combining capacity of the body fluids is probably not greatly inferior to that of the blood.

The length of time required to bring about equilibrium between the blood and the tissues following a change in the alveolar carbon dioxide tension has undoubtedly been greatly underestimated. The rate at which equilibrium is reached is probably a function of the circulation, as reflected by the circulation rate and the degree to which the capillary responses facilitate the exchange of carbon dioxide between the blood and tissues.

## SUMMARY

1. Cats were made to breathe from a gas mixture containing about 11 per cent carbon dioxide until equilibrium had been established at a level which was about 54 mm. of mercury above the normal. During the return to the normal level, while the cat breathed outdoor air, the expired air was collected, and the carbon dioxide which was in excess of that produced by oxidation during the collection period represented that which had been retained by the body as a result of the previous saturation to a higher carbon dioxide level.

2. One kilo of cats' blood, within the carbon dioxide tension limits of these experiments, will retain about 3.2 cc. of carbon dioxide per millimeter of mercury tension difference, while the entire body under the same conditions will retain about 1.78 cc. of carbon dioxide.

3. The carbon dioxide retained by the blood alone was 10.5 per cent of that retained by the entire body.

4. On the assumption that the body is 70 per cent water by weight and that the carbon dioxide retained by the body is held by the water of the body, we have estimated that 1 kilo of tissue fluid, exclusive of the blood, will retain an average of 2.49 cc. of carbon dioxide per millimeter tension difference. In 30 per cent of our experiments the buffering capacity of the tissue fluids was equal to or greater than that of the blood.

5. It requires from 45 to 140 minutes, with an average value of 102 minutes, for the body of the cat to come into equilibrium with a change in the alveolar carbon dioxide tension.

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# WORK AGAINST GRAVITY AND WORK DUE TO VELOCITY CHANGES IN RUNNING

## Movements of the Center of Gravity Within the Body and Foot Pressure on the Ground

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The external work of running may be divided into two parts—1, the movements of the limbs in relation to the center of gravity of the whole body and 2, movements of the whole body as represented by the movements of its center of gravity. I have recently published the results of calculations of the first of these items, namely, the kinetic energy of the limbs at different periods of the running cycle. The measurements on which these calculations were based were taken from a series of cinematographic films of sprinters. The films which were taken with the aid of Mr. C. A. Morrison of the Eastman Kodak Company were run off with exposures of 0.001 second duration at the rate of about 120 exposures per second, and were therefore capable of rather exact analysis. In the present paper I propose to utilize the same data for the calculation of the second of the above items, i.e., the movements of the body as a whole, in order to determine the work involved *a*, in changing the velocity of the body from step to step, and *b*, in raising and lowering the body against gravity. The special significance of the external work in running in relation to the oxygen consumption of the sprinter has been discussed in the previous paper and need not be repeated here except to mention that the sum of all the measured items of external work in running amounted to over 20 per cent of the total energy turnover as measured by the oxygen consumption of recovery.

The movements of the center of gravity of the body may be classified as horizontal, vertical or sideways. The sideways movements could not be observed in the films and must therefore be neglected. The work involved in these sideways movements is certainly relatively small but not altogether negligible. The arms for example carry out considerable sideways movements to help compensate presumably for the sideways movements of the whole body due to swinging the legs. For the present purposes it is necessary to consider only *a*, the horizontal and vertical changes in the position

of the center of gravity inside the body, and *b*, the horizontal and vertical movements of the body itself. From the algebraic sum of the horizontal movements, a and b, and the vertical movements, a and b, the work involved can be calculated.

MOVEMENTS OF THE CENTER OF GRAVITY WITHIN THE BODY. *Method.* It is to the work of O. Fischer (1911) that we owe the analysis of this subject. Since this work is not familiar to most readers it seems advisable to give a brief description of his method.

In figure 1 let $m_1$ represent the body with its center of gravity at $S_1$ and let $m_2$ represent a limb with its center of gravity at $S_2$. The common center of gravity $S_0$ must lie then on the line connecting $S_1$ and $S_2$ in such a position that $S_1S_0 : S_0S_2 = m_2 : m_1$. If $S_0H_1$ is drawn parallel to $S_2O$ then it is obvious that the point $H_1$ divides $S_1O$ inversely as $m_1 : m_2$. In other words, the point $H_1$ would be the center of gravity of a system consisting of a mass $m_1$ at $S_1$ and a mass $m_2$ at $O$. Such a system was called by Fischer the "reduced system" with respect to $m_1$. The point $H_1$ he called the "principal point" (Hauptpunkt) of this system and the distance $H_1S_1$ he called the "principal interval" (Hauptstrecke). Likewise if $H_2$ is drawn parallel to $S_1O$, then $H_2$ is the principal point of the reduced system with respect to $m_2$ and would be the center of gravity of a system in which $m_2$ was located at $S_2$ and $m_1$ at $O$. From the diagram (fig. 1) it is obvious that in order to find $S_0$, knowing the position of $H_1$ it is only necessary to lay off a line $H_1S_0$ in a direction parallel to the axis of $m_2$ and of a length equal to $H_2O$, the principal interval of $m_2$. This is the method by which the movements of $S_0$ can be followed as the angles of the limbs change.

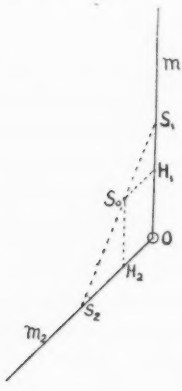


Fig. 1. Construction showing the centers of gravity, $S_1$ and $S_2$ of the body and upper leg, $m_1$ and $m_2$.

When there are many limbs attached to $m_1$, then the principal point of $m_1$ would be the center of gravity of the system consisting of a mass $m_1$ at $S_1$ with the masses of all the lower limbs concentrated at $O$ and the masses of all the upper limbs concentrated at the shoulder joint. Likewise the principal point of each member of the system is determined from the center of gravity of the reduced system related to that member. The reduced system related to the upper arm consists of the mass of the upper arm at its center of gravity, the mass of the lower arm at the elbow joint and the mass of the body plus all the other members of the body at the shoulder joint. The center of gravity of three such masses so located would be the principal point of the upper arm. The principal interval is the distance between that point and the shoulder joint. The principal interval is always measured toward the member the principal point of which is chosen as reference. Thus to find the center of gravity of the whole body when the principal intervals of all the members and the angles of all the limbs are known one starts from the principal point of the body and lays off each principal interval in succession in the proper direction, the second one beginning at the end of the first, etc. The point finally arrived at by this construction is the center of gravity of the whole system.

From measurements on cadavers Fischer (1911) has given data from which it is possible to estimate the principal intervals. These are always certain fractions of the lengths of the limbs, as given below.

| | | | |
|---|---|---|---|
| Upper arm | 0.047 | Upper leg | 0.122 |
| Lower arm | 0.021 | Lower leg and foot | 0.043[1] |

The lengths of the limbs are measured from joint to joint, the lower arm from the elbow to the wrist and the lower leg from the knee to the ankle. I have usually measured the lower leg from the knee to the sole of the foot in which case the principal interval may be taken without serious error as 0.035 instead of 0.043 of the length of the limb.

The actual procedure in determining the center of gravity from the cinema films is as follows: The angles of the upper and lower arms and legs are first measured from the films when projected on a screen, one measurement being made at every second frame. This is done simply by laying a protractor along the long axis of the limb and reading the angle by means of a plumb line. These angles are then plotted for each limb against the frame number (i.e., time) and smooth curves are drawn through the points in such a manner that they return to the starting point at the end of the running cycle. Corrected angles are then read off from the graphs and are used in laying off the principal intervals. This is done on graph paper laid off in millimeters after the manner illustrated in figure 2 for runner 7. The principal intervals in this case were 1.46 and 0.86 cm. for the upper and lower arms respectively and 5.0 and 1.98 cm. respectively for the upper and lower legs. This construction has been carried out in figure 2 for 16 different points during the running cycle. Every fourth frame (or exposure) on the film was used for calculation. In the case of runner no. 7 the camera was being turned at such a rate that each frame represents 0.0079 second so that the center of gravity is followed every 0.0316 second throughout the run. The whole cycle is represented by 64 frames or 0.506 second. (Point 17 in figure 2 if represented would correspond exactly to point 1.)

From the construction of figure 2 it is possible to determine the contribution made by any part of the body to the vertical and horizontal movements of the center of gravity. Take the construction of point 1 for example. Having laid off the principal intervals for the eight different members in the proper directions one arrives at a point which is 13 cm. below the origin of the construction and 0.1 cm. in front (to the left as drawn). For point 8 on the other hand it is found that the presence of the limbs at the observed angles has lowered the center of gravity 15.2 cm. below the principal point of the reduced system related to the body and

[1] Fischer gives 0.04 for the lower leg without the foot.

moved it 1.0 cm. in front of the principal point. In a similar manner the separate effect of each arm and each leg on the center of gravity has been determined and the results, as far as the vertical movements are concerned, are plotted in figure 3.

*Vertical movements of the center of gravity.* In figure 3 point 1 (fig. 2) begins at the left and other points are plotted in order to the right. Ordi-

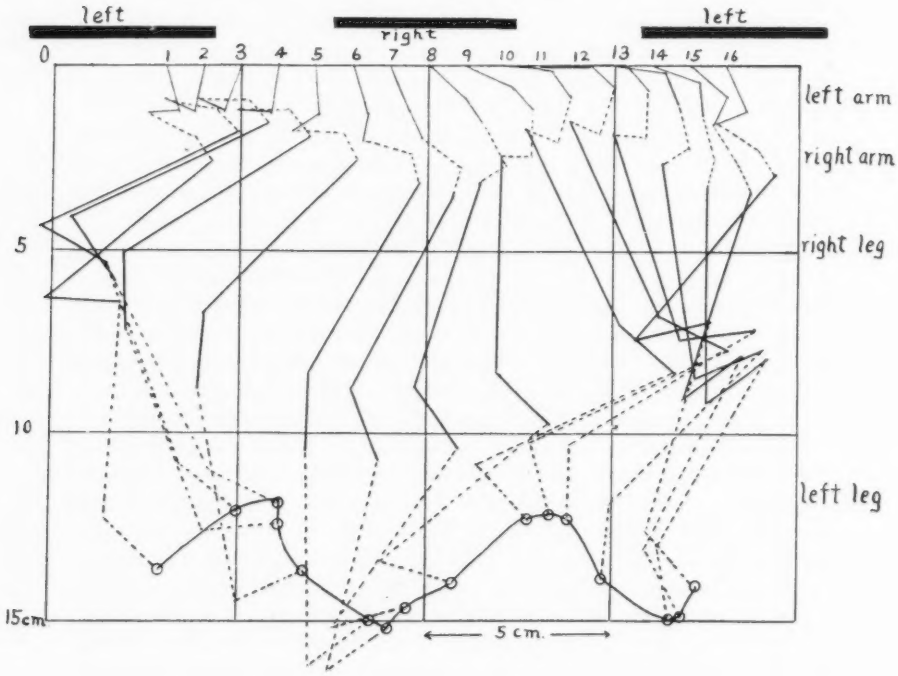


Fig. 2. Construction for the determination of the movements of the center of gravity within the body due to limb movements at 16 different phases of one running cycle of runner 7.

nates in this case represent in centimeters the distance of the center of gravity below the principal point of the body. This principal point is located according to Fischer (1911) on the longitudinal axis of the body at a distance above the hip joints equal to 0.451 of the distance between the hip joints and the shoulder joints. The graphs show that the movements of the two arms are such that they both tend to raise the center of gravity of the body more or less synchronously. These maxima for the two arms do not always coincide exactly since the forward and backward

swings are not entirely symmetrical. Adding together the separate effects of the right and left arms, we find the curve for both arms which rises and

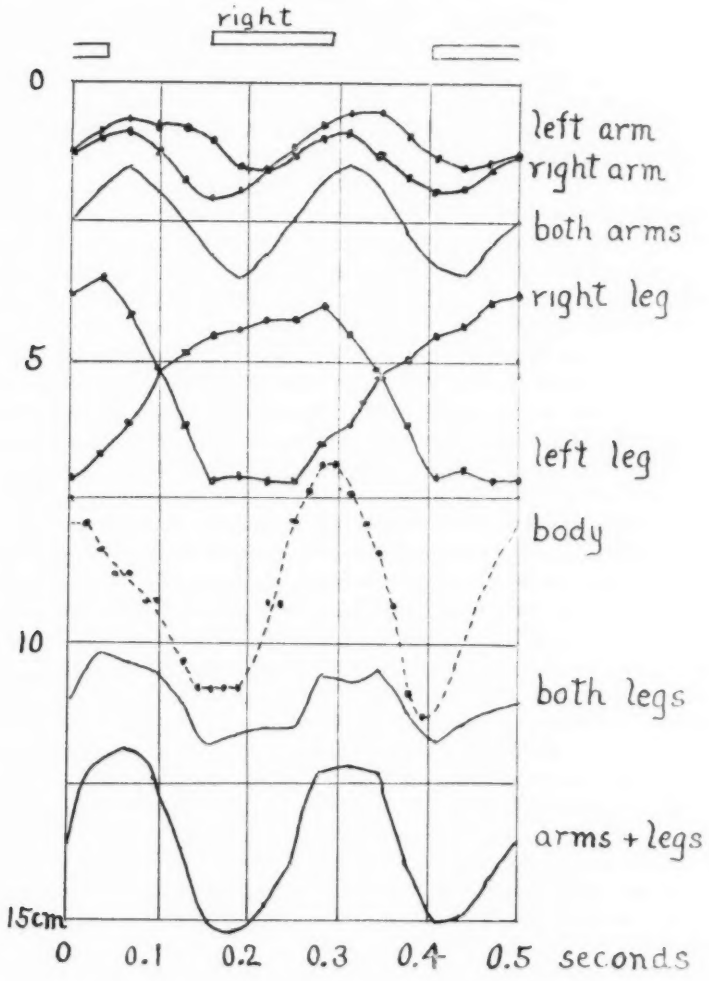


Fig. 3. Vertical movements of the center of gravity within the body during one running cycle. Runner 7.

falls twice in each complete cycle. The effects of the legs separately are plotted next below and it can be seen that during the period when the right

foot is on the ground its effect in lowering the center of gravity is at a maximum, i.e., the curve passes through a minimum. These individual oscillations are rather large but happily the movements of the two legs tend to compensate for one another. In this respect the legs differ from the arms. The oscillations of the curve for both legs together are much smaller than for either leg separately. It might be expected that the movements of the arms would tend to compensate for the movements of the legs. This, however, is not the case for vertical movements, for the rises in the curve for both legs are exactly synchronous with the rises in the curve for both arms. The lowest graph of figure 3 represents the effect of both arms and legs combined.

It was of special interest to compare this rise and fall of the center of gravity of the body with the observed rise and fall of the body itself. The dotted graph of figure 3 represents the measurements made directly from the film of the height of the top of the ear above a certain horizontal line. The actual position of this curve in the figure is entirely arbitrary but the relative changes in height are comparable. It is striking that *the whole body rises synchronously with the rise of the center of gravity within the body.* This rise reaches its maximum just at the end of the period of foot contact as shown in the figure. The actual height of the rise is equal to the sum of the two effects (about 7.4 cm.) which are not very different in magnitude.

It is instructive to correlate the height of this rise, with the distance the runner would fall if his period in the air were a real jump. From the film it appears that the runner was off the ground in this case for 10 frames or 0.079 second. If during half of this time or 0.0395 second he was a freely falling body he must have fallen $\frac{1}{2}$ $gt^2$ or $980/2 \times 0.0395^2$ or 0.76 cm. This is only about $\frac{1}{10}$ of the total rise (7.4 cm.) as measured from the film and calculated from the positions of the limbs. Reference to figure 3 shows that all of the rise of the center of gravity within the body and all of the rise of the body as a whole takes place during the period of foot contact rather than between contacts. On the whole therefore the runner does not leap into the air, at least not more than 0.76 cm. Instead *he raises himself and his center of gravity within his body while his foot is on the ground and then permits his body to fall while he is in the air.* If he is considered as falling throughout the period in the air he would fall $980/2 \times 0.079^2$ or 3.06 cm. The rest of the total fall of 7.4 cm. takes place after the foot touches the ground.

In order to confirm these facts concerning the time relations of the vertical movements of the body, further data from other runners have been collected in figure 4. The seven upper graphs (solid lines) were taken from measurements on as many different runners and represent the vertical movements of the whole body as measured from the height of the ear or of a black spot fixed on the neck (no. 21). The ear offers a fairly sharp point

for measurement and does not vary in height with movements of the head as much as the top of the head or the nose. The measurements are not wholly free from the possibility of this error, however. The eight lower graphs (dotted lines) represent the calculated movements of the center of gravity within the body due to the varying positions of the limbs. Frame 30 in each of the fifteen graphs represents the moment when the toe leaves the ground. The moment when the foot makes contact with the ground varies in different runners and is indicated on each graph by a small arrow. Each graph represents one complete cycle. Since the lengths of these cycles vary the second contact block cannot apply to all the runners and must be considered as approximate only. Abscissae represent frames on the films since the film speed varied slightly in different runners.

Inspection of figure 4 shows that on the whole the rise of the whole body as well as the rise of the center of gravity within the body reaches a maximum at the moment when the toe leaves the ground. With few exceptions therefore the center of gravity is falling in relation to the ground throughout the period between contacts. It may also be seen that all of this fall, particularly the fall of the center of gravity within the body, is not complete before the foot again makes contact with the ground, i.e., the curves continue to fall slightly after the points marked by arrows.

From the data of figure 4 it is possible to calculate the work done against gravity in running. The average rise of the body as a whole from the seven upper graphs is 3.12 cm. and the average rise of the center of gravity within the body from the eight lower graphs is 2.92 cm. The sum of these is 6.04 cm. If the weight of the runner is 68 kgm. and the length of the cycle is 0.5 second then the body is lifted $6.04 \times 4$ or 24.16 cm. per second. The horse power is then $\frac{68 \times 24.16 \times 980}{746 \times 10^4} = 0.215$ horse power.

In my previous paper I estimated this factor as only 0.1 horse power, thereby neglecting the rise of the center of gravity within the body. This is perhaps safer for the kinetic energy of the limbs is sufficient in most cases to raise the limb the requisite distance against gravity. There is therefore danger of counting the same work twice if the work of raising the limbs is included in the total.

A number of previous estimates have been made of the work done against gravity in human progression. Some of these investigations were concerned with the problem of walking only (Braune and Fischer, 1894; Pottevin and Faillie, 1925). With subjects running on a moving belt Benedict and Murchhauser (1915) observed a rise of the body of 7.56 cm. per step. This is definitely higher than my figure for free running, and higher than the figure of 3.3 cm. rise per step calculated from Demeny and Marey, 1885, who report 2.3 kgm. of work per step due to vertical oscillations at a velocity of about seven meters per second. Their value for slow running corresponds better with that of Benedict and Murchhauser.

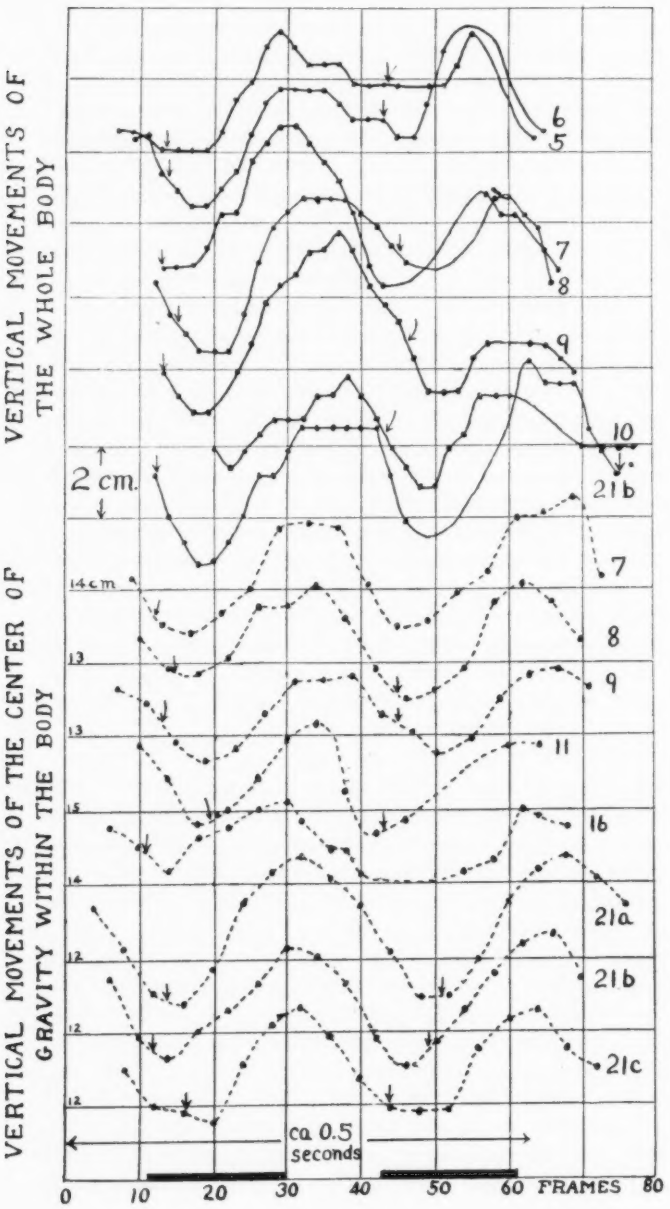


Fig. 4. Vertical movements of the center of gravity within the body and of the body itself. In each graph frame 30 is the point when the toe leaves the ground. In other points the contact blocks are approximate only. The graphs cover one complete running cycle in every case. Small arrows indicate points when the foot makes contact with the ground where this could be observed. One-half second is represented by 74 frames in the case of no. 21 and by 62.5 frames in all others. Each square vertically represents 2 cm. but the curves are arbitrarily spaced. Absolute values for the lower eight curves may be obtained by reference to the figures at the left of the diagram which give the absolute value of the ordinate marked for the eight different curves in order. This is the vertical distance in centimeters between the principal point of the body and head and the center of gravity of the whole man.

HORIZONTAL MOVEMENTS OF THE CENTER OF GRAVITY. Applying further the method of Fischer, as examplified in figure 2, we may consider the horizontal movements of the center of gravity within the body which are produced by the varying angles of the limbs. As in the case of the vertical movements, the contributions of the various members to the forward and backward movements are read off from the chart and are plotted singly and in combinations in figures, 5, 6, and 7. In these charts a rise of the curve (positive values) indicates that the center of gravity is moving forward in the body and vice versa. Comparing the curve for the right leg in figure 5 with the period of right foot contact as indicated at the top of the chart it is obvious that while the foot is on the ground it tends to throw the center of gravity of the body more and more backwards. Simultaneously, however, the left leg is thrown far forwards so that the effects of the two legs are more or less compensatory and the variations due to both legs, as indicated by the dotted line, are relatively small. On the whole in this runner the legs tend to keep the center of gravity somewhat further forward than in the normal standing position. The two arms, however, have the reverse effect, as shown in figure 6 by the dotted line. In this runner it is also found that the movements of the arms tend to compensate somewhat for the movements of the legs so that when the combined effects of all the limbs are plotted the variations are even less than with either arms or legs separately.

That this runner is not altogether typical, however, is shown by the curves of figure 7 from a faster runner, 21, who ran with a mean velocity of 8.27 meters per second while no. 7, plotted in figures 5 and 6, attained a mean velocity of only 7.68 meters per second. Whether or not the differences to be noted are related to the better speed of no. 21 cannot be stated. It may be observed, however, that the average combined effect of his legs throws the center of gravity rather further forward than no. 7 and the combined effect of his arms throws the center of gravity rather less far backwards than no. 7. The final result is that the limbs as a whole keep the center of gravity 1 to 2 cm. ahead of its standing position while in no. 7 it is if anything a little behind this position. It will be noticed also that no. 21 compensates with the movements of his arms more accurately so that the dotted graph representing the effects of the two arms combined shows hardly any oscillations.

COMBINED MOVEMENTS OF THE CENTER OF GRAVITY IN A SAGITTAL PLANE. It is perhaps interesting to consider the movements of the center of gravity as a whole, sideways movements being neglected. For this purpose data from 6 runners are collected in figure 8, vertical movements in cm. being plotted as ordinates, and horizontal movements as abscissae, in such a way that forward movements are indicated by a plus sign and are plotted to the right. The graph in each diagram follows the successive positions occupied by the

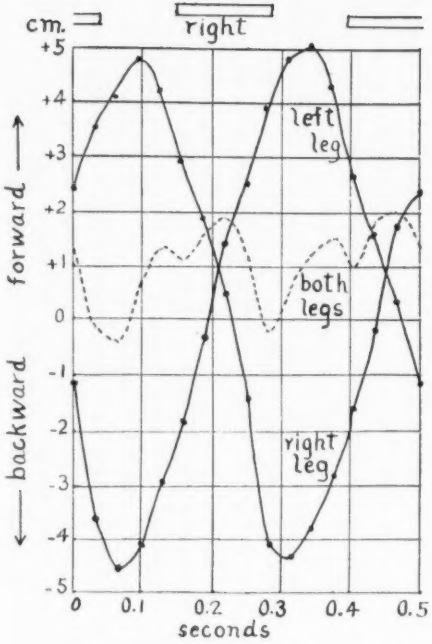


Fig. 5. Forward and backward movements of the center of gravity of runner 7 due to the legs.

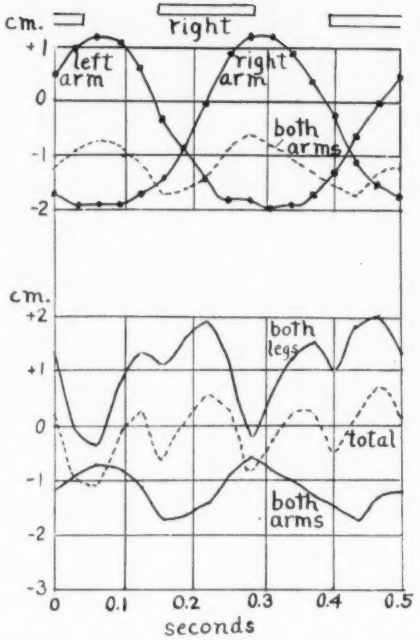


Fig. 6. Similar to figure 5 for arms and legs combined of runner 7.

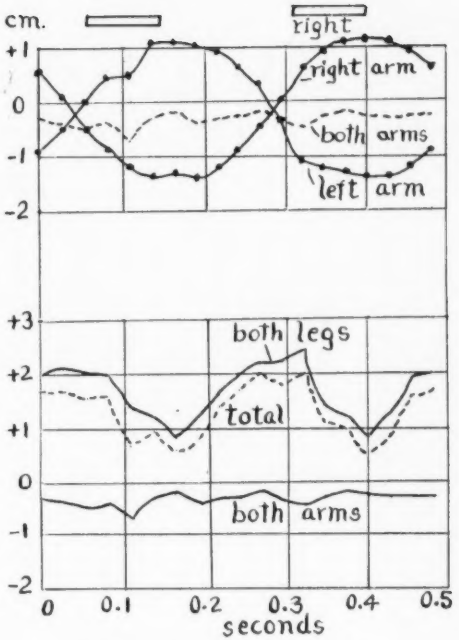


Fig. 7. Similar to figure 6 but data taken from runner 21.

center of gravity at intervals of four frames or about 0.032 second. These positions are numbered consecutively in order to assist in following the curve. In the case of no. 7 the center of gravity evidently describes two figures of eight in each cycle. The other graphs show that in general the center of gravity moves diagonally upwards and backwards and then re-

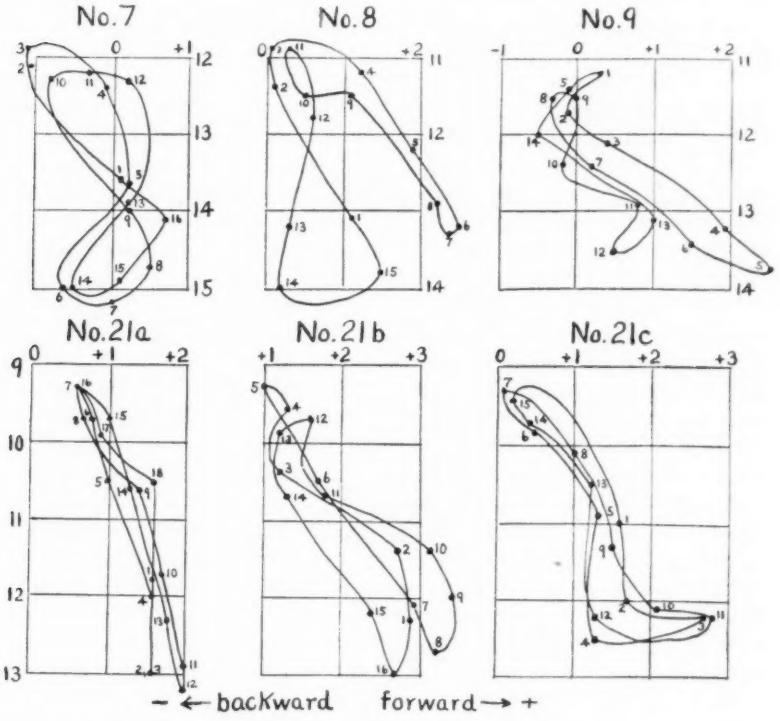


Fig. 8. Sideways projection of the movements of the center of gravity within the body due to the movements of the limbs during one running cycle. Points are numbered consecutively and represent equal intervals of four frames or 0.032 second (0.027 sec. for no. 21). Each square represents 1 cm. both vertically and horizontally.

verses and goes forwards and downwards. It will be noticed that nos. 7 and 9 are the only ones in which the center of gravity passes through any negative positions. It may be remarked that no. 9 was one of the slowest runners with an average speed of only 6.91 meters per second, no. 8 having a speed 7.22 meters per second and no. 21 attaining velocities of 8.45, 8.27 and 8.50 meters per second in three different sprints.

HORIZONTAL MOVEMENTS OF THE WHOLE BODY. It is obvious that one factor in the efficiency of a runner will be the uniformity with which he maintains his velocity. He must necessarily lose some velocity while he is free in the air because of the wind resistance and this must be made up when his foot next touches the ground. This by itself is a small item, but there is in addition a possibility of some loss of momentum when the foot strikes the ground. The magnitude of the energy lost in this way has never been satisfactorily estimated to my knowledge.

To obtain evidence on this point measurements have been made upon runners in two ways: 1, the successive positions of the tip of the nose or of a marker tied around the neck have been followed to determine the velocity changes of the upper part of the body; and 2, the movements of a marker tied tightly around the waist have been followed to determine the velocity of a fixed point on the body as close to the true center of gravity of the body as possible. These markers were pieces of cardboard fixed securely to stiff wire frames which were braced against the back both in an up and down and in a sideways direction. It is possible that there were nevertheless some vertical movements not representative of the movements of the body as a whole but the horizontal movements could not have been distorted appreciably. In some cases measurements of the position of the body were made at every frame. The velocities were then calculated for intervals of 2, 4, or more frames in various combinations. In spite of the short exposures used the blurring was sufficient so that the error in each such velocity determination was about 5 per cent for a four-frame interval. The results of these observations for four different runners are plotted in figure 9. The solid lines represent the velocities of the pelvis and the dotted lines the velocities of the neck (or nose in no. 30). The results show with striking uniformity that the body tends to rock backwards and forward slightly during the run. It will be found that the velocity of the waist (solid line) is always higher than that of the neck during the period of contact, and vice versa while the body is in the air. This is natural, for the body is being pushed forward from the hips while the foot is on the ground and the head follows with a slight lag due to the rigidity of the trunk. This rule is followed with perfect uniformity in every runner (over 20 in number) whom we have measured, but these four cases will suffice for its demonstration.

If the maximum and minimum velocities of the center of gravity of the runner are known the work involved can readily be calculated. For this purpose it would seem to be most nearly correct to take the maximum and minimum velocities of the waist, for this point is surely most representative of the movements of the center of gravity of the body itself. Figure 9 shows, however, that these changes in velocity are impossibly large, i.e., from 7 to 8 meters per second, which would correspond to about 2.6 horse

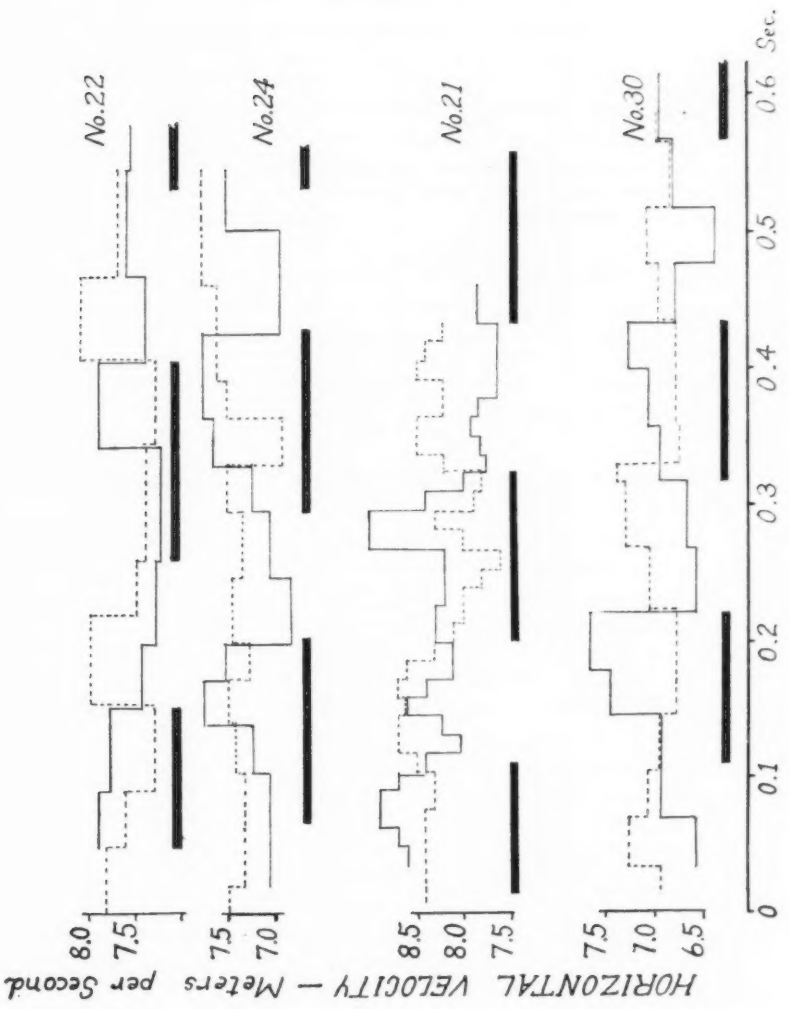


Fig. 9. Horizontal velocities of the neck (dotted line) and of the pelvis (solid lines) of four runners.

power. It seemed therefore probable that the movements of the center of gravity within the body must be of such a direction and magnitude as

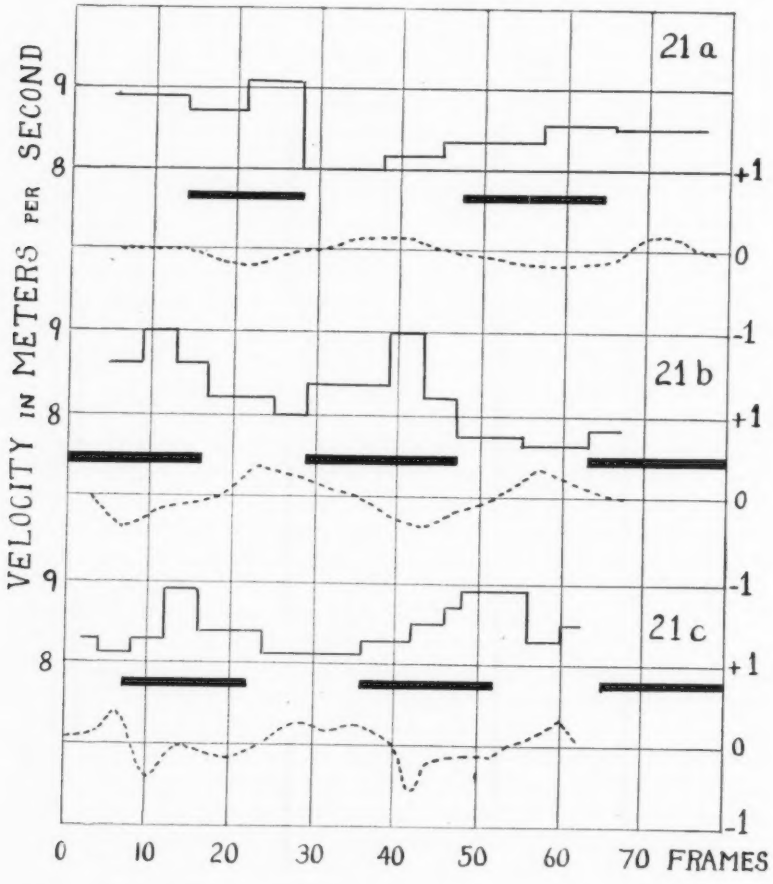


Fig. 10. Graphs showing that when the observed forward velocities of the pelvis are high (solid line) the center of gravity (dotted line) has a backwards velocity, as represented by negative values. Each square vertically represents a velocity change of one meter per second. Figures at the right refer to the dotted lines. One second equals 149 frames in the film of no. 21a and 129 frames in b and c.

to compensate for these apparent changes in velocity. That this is indeed the case is shown by the graphs of figure 10. The solid lines represent the observed waist velocities of no. 21 in his three runs. The dotted lines repre-

sent the horizontal velocities of the center of gravity within the body of the same runner. These latter figures were obtained graphically by taking the slopes of curves such as those of figures 6 and 7, representing the forward and backward movements of the center of gravity due to the combined effects of all the limbs. These center of gravity velocities are plotted in the same units as the waist velocities but their relative positions are arbitrary. It can be clearly seen that *when the waist velocity is high the center of gravity is moving backwards (plotted downwards) and vice versa.* This indicates that the changes of velocity of the center of gravity in relation to the ground are not so great as the variations in velocity of the waist would indicate. When the limbs are carried forward while the body is in the air some other part of the body must move backwards a corresponding amount for in a system like the body, when freely suspended, no movement within the system can move the center of gravity of the whole system.

## DIRECT DETERMINATION OF THE WORK ASSOCIATED WITH CHANGES IN VELOCITY OF THE BODY BY MEANS OF A RECORDING PLATFORM

(With the assistance of W. B. Latchford, A. Petrilli, C. I. Wright and C. A. Morrison)

It is theoretically possible to take the algebraic sum of the waist velocities and the center of gravity velocities and so to determine the true velocity of the center of gravity within the body. The determinations of the waist velocity are, however, themselves subject to considerable error and the assumptions involved in the determination of the velocity of movement of the center of gravity within the body are numerous and hardly negligible. The forward and backward tilting of the body demonstrated by the differences between the waist and head velocities (fig. 9) for example have been neglected. The values of the principal intervals themselves are approximations. Proper account could not be taken of the weight of the clothing, particularly of the rubber-soled shoes worn by the runners. It is doubtful therefore whether such a procedure would mean anything after it was completed. Casual inspection of the curves (fig. 10) suggests, however, that the velocity changes of the waist are greater than can be accounted for completely by the changes in the velocity of the center of gravity.

Fortunately it has been found possible to avoid all these difficulties concerned with velocity measurements by attacking the problem with an entirely independent method. Velocity changes can be caused only by contact with some object external to the body itself. The only external objects which can be encountered in this way are the air and the ground. If the ground offers any resistance this must mean that the foot pushes forward on the ground when contact is made. Moreover the backward

push on the ground when the toe leaves must be a measure of the total external resistance encountered by the runner, i.e., the sum of the air resistance and the ground resistance. For these reasons it seemed (Aug., 1928) important to measure directly the horizontal components of the foot pressure exerted on the ground during running. The results obtained so far by this method must be regarded as somewhat preliminary in nature but further measurements have been unavoidably interrupted for a time and it seems necessary to report the present status of the work in connection with the analysis of the moving pictures.

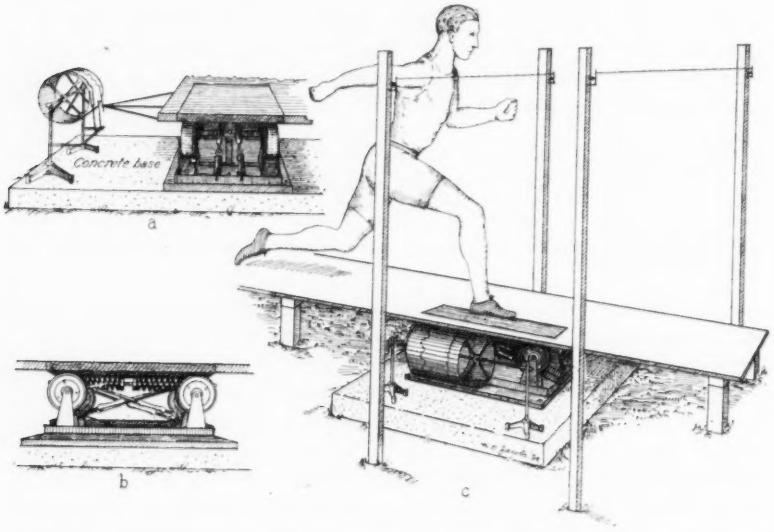


Fig. 11. Arrangement of the apparatus for recording the forward and backward components of the pressure of the foot against the ground.

*Method.* The direct method depends upon the measurement of the horizontal pressure exerted by the foot while in contact with the ground. A platform (*trottoir dynamicque*) was built for a similar purpose by Amar (1916 and 1919) who measured both horizontal (backwards) and vertical pressures. He used the apparatus chiefly for the study of walking and I have been able to find only a single tracing of the horizontal pressures obtained with this apparatus in running. This single diagrammatic record (cf. Amar, 1923, p. 525, also 506) showed that at the moment of contact the foot pushes forward on the ground, and later of course backwards. Unfortunately no information was given concerning the velocity of the runner nor his weight so that the energy change cannot be determined. It should

be mentioned in addition that Marey (1892) has used a dynamographic platform for measuring vertical pressure on the ground and Braune and Fischer (1899) have calculated from their moving pictures the direction and magnitude of the foot pressure on the ground in walking.

The apparatus built by Amar involved the use of rubber bulbs which were compressed by the movements of the platform and recorded on a drum by tambours with air transmission. For more reproducible and quantitative results it seemed better to use direct mechanical recording. The diagram of figure 11 is more or less self-explanatory. The platform used was 13 inches long and 9 inches wide, of heavy oak. It was mounted on wheels with hard rubber tires screwed to an iron frame which was bolted to a concrete block set in the ground. The platform was held in position by four diagonal springs with turnbuckles for adjustment and two horizontal springs. The diagonal springs provided a small vertical component which prevented the platform from oscillating up and down. The drum was most conveniently turned one revolution by hand at the moment when the runner passed, time being recorded by a tuning fork. The platform was calibrated by setting a screw in the top and pulling on it with a spring balance, simultaneously recording the deflection of the lever on the drum and the reading of the spring balance. As used for most of the records, a pull of 31.3 kgm. caused a deflection of 1 cm. on the drum, the deflection being strictly proportional to the tension. The platform is connected to the lever by three flat brass strips fastened underneath the platform and so arranged in diagonal positions that horizontal oscillations with respect to the platform are practically impossible. On the tip of these three brass strips is a small horizontal pin which fits into a hole on one arm of the lever. The recording arm of the lever was long enough, about 5 cm., to magnify the movements of the platform about two times. The actual movement of the platform in running is therefore only a few millimeters backwards and forwards. The runner does not notice that it is any different from the solid board walk upon which he runs. The actual track is longer than that shown in figure 11, so that the runner is not disturbed as he passes the recording platform, by the immediate necessity of jumping off.

The velocity is recorded by two light threads stretched across the track at the level of the shoulder. When these are encountered an electric switch is opened which releases a signal magnet on the drum. Sixteen sample records obtained with this apparatus are shown in figure 12. There is some tendency of the platform to oscillate but this is largely damped out by the body itself. The natural period of the empty platform is 0.028 second for one complete vibration. In each of these records of figure 12, as is true more or less clearly in all of the 41 records which we have obtained, there is an initial downstroke indicating a forward push on the ground, followed by an upstroke as the runner takes off. The lower two

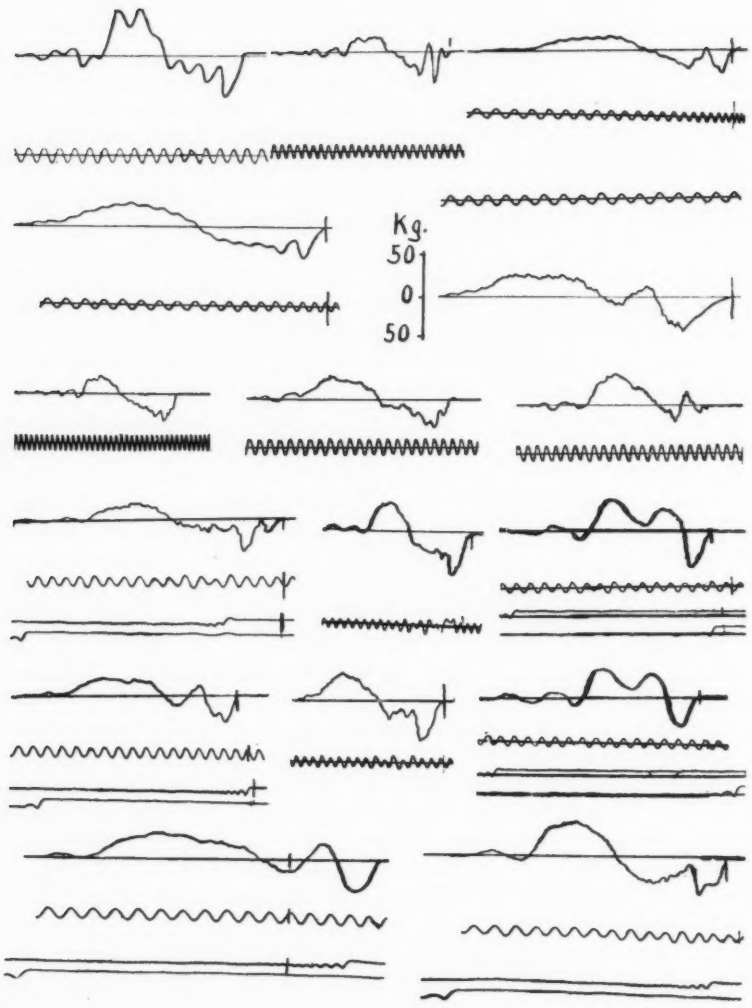


Fig. 12. Records obtained with the recording platform. A downstroke on the upper graph in each case represents a forward push against the platform and vice versa. The tuning fork vibrates 100 per second. The two signal magnets present in six of the tracings record the time necessary to run one meter. Reduction 0.6 of the original size.

lines in six of these records represent the signal magnet tracings, the time interval being the time necessary to run 1 meter. The velocity in all these cases was between 6.4 and 7.7 meters per second.

*Calculation.* The drum record of these experiments represents force plotted against time. The area under the curve therefore represents momentum. During the forward push on the ground the area represents momentum lost and during the backward push the area represents momentum gained. Assuming that the particular step recorded is representative of the whole series, then the difference between the momentum gained and the momentum lost is the momentum lost while the body is in the air, i.e., momentum lost by wind resistance. To measure these force-time areas the graphs were thrown upon a screen by an epidiascope and were then traced. The areas so traced were cut out and weighed. Thus the area was expressed in gram seconds.

The calculation of the energy loss is carried out as follows: Let $u$ and $v$ be the original and final velocities of the runners, let $m$ be his mass and $Ft$ be the area under the drum record.

Then

$$mu = Ft + mv \quad \text{or} \quad u = \frac{Ft}{m} + v$$

$$u^2 = \frac{F^2t^2}{m^2} + \frac{2Ftv}{m} + v^2$$

$$\frac{mu^2 - mv^2}{2} = \frac{F^2t^2}{2m} + Ftv \tag{1}$$

This last equation gives the energy change in terms of the mass of the runner, the area under the drum record and the velocity of the runner as recorded on the drum by the signal magnets. (The velocity actually recorded is probably somewhere between $u$ and $v$.) In order to express this energy in terms of horse power it is necessary to know the number of steps per minute. This could be determined with a stop watch but where such data were lacking an average value could be assumed without serious error from data on other runners.

*Results.* When the final results were expressed in horse power they were plotted as frequency curves in figure 13. In this figure, A represents the forward pressure on the ground in 41 cases, and B the backward pressure in the same cases. Average values show that for the whole run, energy is wasted at the rate of 0.34 horse power on the forward push and is gained at the rate of 0.5 horse power on the backward pushes. This result would indicate that the wind resistance required an energy expenditure at the rate of 0.5 − 0.34 or 0.16 horse power. That this is a reasonable

figure for wind resistance at the velocities attained by our runners is shown in table 1, taken from A. V. Hill (1928) and including for comparison our own value and the values calculated from the observations of du Bois-Reymond (1925), for some of the velocities studied by Hill. Hill measured directly the wind pressure on a miniature man in a wind tunnel and so

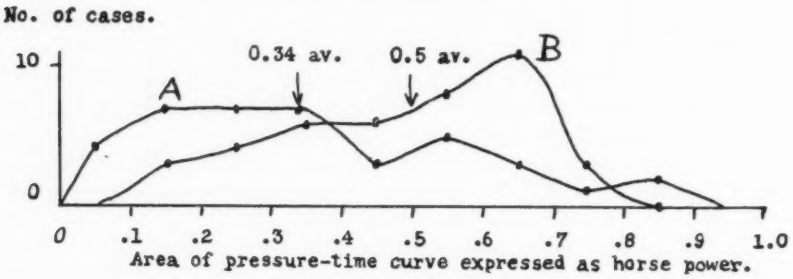


Fig. 13. Frequency curves showing the distribution of values obtained for the forward push A, when the foot makes contact with the ground and the backward push B when the runner takes off. The results are expressed in terms of horse power lost and gained respectively.

calculated the energy requirement. DuBois-Reymond used a similar method but a larger model man. He found an average figure of 632 grams pressure for the clothed figure at a velocity of 5 meters per second. He also measured the pressure on the naked figure and in many different positions of the body. Assuming that the wind resistance varies as the

TABLE 1

*Power expended in overcoming wind resistance at different speeds*

| METERS PER SECOND | HORSE-POWER | | |
|---|---|---|---|
| | duBois Reymond, 1925 | Hill, 1928 | Fenn |
| 9.85 | 0.317 | 0.32 | |
| 7.74 | 0.154 | 0.16 | |
| 6.4–7.7 | | | 0.16 |
| 6.98 | 0.113 | 0.12 | |
| 6.44 | 0.089 | 0.09 | |

square of the velocity the figures of table 1 have been calculated and they show a striking agreement with the measurements made later by A. V. Hill, without apparently any knowledge of this earlier work on the subject.

The figure obtained from the measurements on the platform as shown in table 1 evidently fits into Hill's series satisfactorily. It cannot be regarded as a highly precise value of course, because the variation in the 41 records

which were averaged together was so great. It is evidently impossible to make every step in a run like every other one, particularly when one is endeavoring to gauge one's steps in such a way that one of them will be sure to fall directly on a particular spot. This difficulty constitutes in fact an obvious and important criticism of the results. On account of it, the measurements were given up altogether until another platform could be built large enough to be hit regularly without special thought. It is, however, striking that every one of our records showed some indication at least of an initial forward push. Our subjects found it impossible to run without giving this forward push. It is conceivable that a good sprinter would be able to do this. We believe however that the result obtained represents the performance of an average runner, who must therefore expend energy at the rate of 0.5 horse power during a fast sprint to main-

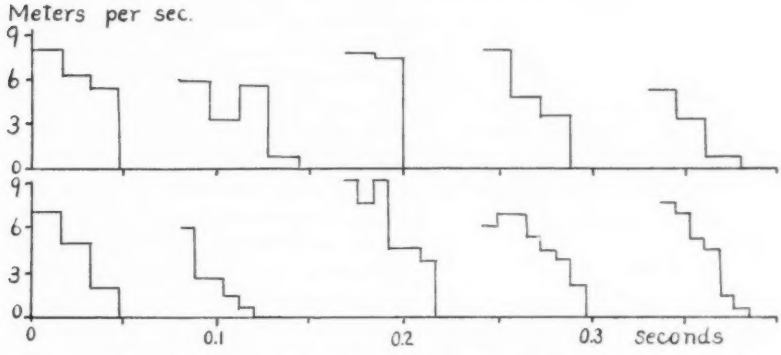


Fig. 14. Forward velocities of the heels of ten runners just before the foot makes contact with the ground, when its velocity becomes zero.

tain his mean velocity. This would mean that his velocity would diminish from say 7.7 to 7.5 meters per second at each step or twice during each running cycle. Referring again to figure 10 it will be seen that this variation is much less than the change in velocity of the waist which was actually observed and is quite consistent with the small residual change in velocity which might be left after correcting for the velocity changes of the center of gravity within the body. It is so small that it would be impossible to measure it with certainty by the round-about method of correcting the waist velocity in relation to the ground by the center of gravity velocity in relation to the body.

How the foot makes contact with the ground. The graphs of figure 14 lend further support to the belief that the forward velocity of the center of gravity of the whole body in relation to the ground is checked

slightly when the foot comes in contact with the ground. These graphs represent the forward velocities of the heels of ten different runners just before the foot came into contact with the ground. These velocities are seen to diminish gradually from the mean velocity of the body, which is between 7 and 8 meters per second, to zero. At no time previous to contact is the foot moving backwards in relation to the ground. In some cases the forward velocity of the heel seems to diminish very suddenly which can only be accounted for by a sudden forward push on the ground. Some of the graphs are not inconsistent with a zero velocity of the foot at the moment of contact. It is impossible to be very certain of this point because one cannot tell the exact moment of contact with a greater accuracy than one frame. Some runners come down a little on the side of the foot which makes it still more difficult to determine certainly whether contact has been established. Practically all of the runners come down flat footed or slightly on the heel even at top speed. Runner 21 makes contact sometimes with the ball of the foot and sometimes with the whole foot. At the moment of contact the foot is of course going backwards in relation to the body for its forward velocity is decreasing, while that of the body remains practically constant.

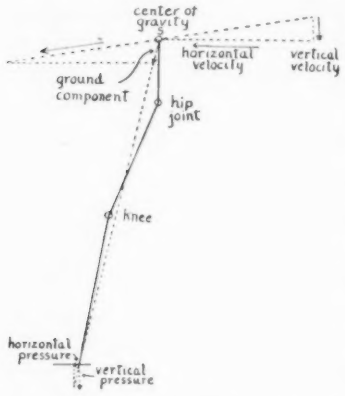


Fig. 15. The leg and the center of gravity, $S$, of the body at the moment when the foot makes contact with the ground. Construction shows the method of calculating the forward pressure on the ground from the velocity and angle of contact. The distance from the hip to the center of gravity is about twice too large in this drawing.

CALCULATION OF THE LOSS OF MOMENTUM FROM THE ANGLE OF CONTACT WITH THE GROUND. Further consideration of the exact positions of the legs at the moment of contact with the ground permits an approximate calculation of the maximum amount of forward pressure on the ground by still another method. This indirect and entirely independent method yields results which agree very well with those measured directly on the platform. It is free from some of the objections of the platform method because it can be applied to our fastest runner when running without thought of trying to hit the platform. There is no possibility therefore of any artificiality due to this cause.

The method is illustrated in figure 15. The solid lines represent the positions of the upper and lower legs and the longitudinal axis of the body be-

tween the center of gravity and the hip joints at the moment of foot contact with the ground. A dotted line is drawn from the center of gravity, *S*, to the point of foot contact. (The center of gravity is determined by the method already outlined.) This line makes an angle of 79° with the horizontal. Since the point of contact is ahead of the center of gravity it is obvious that there should be some forward component in the foot pressure on the ground. The center of gravity makes contact with the ground through a two-joint system with the joints more or less fixed by muscles. It is as if at the moment of contact a body of mass $m$ and velocity $u$ impinged upon the top of this two-joint system. The direction and velocity with which it strikes can be estimated from the horizontal velocity, say 7.5 meter per second, and the vertical velocity. The latter is calculated from the average figure of 0.1 second for the period between foot contacts in 16 runners. If the runner has been falling freely for this length of time then his vertical velocity at the moment of contact is $gt$ or 0.98 meter per second. The resultant of this velocity and his horizontal velocity may be found graphically or by calculation and can in turn be resolved into a horizontal component and a "ground component" (see fig. 15) directed along the line connecting the center of gravity with the point of contact. This ground component in turn may be resolved into a horizontal and a vertical component at the point of contact. When the construction is carried out or the corresponding calculation made for a series of fourteen runners the following result is obtained (cf. also fig. 16).

| | | *Average* |
|---|---|---|
| Angle of contact in degrees | 76, 70, 72, 80, 77, 80, 80, 79, 72, 77, 73, 79, 80, 76 | 76 5 |
| Forward pressure component, cm./sec. | 25, 36, 32, 17, 23, 17, 17, 19, 32, 23, 30, 19, 17, 25 | 23 7 |

The average forward pressure component of the velocity is therefore 0.237 meter per second when the initial velocity is 7.5 meters per second.[2] The momentum which is lost by forward pressure on the ground is therefore 0.237 $m$. This should be equal to the force time area, $Ft$, recorded on the drum by the platform. The average of all the forward pressure component areas so recorded corresponded to 0.34 horse power or a change of velocity from 7.5 to 7.37 meters per second. The platform records therefore a loss of velocity of 13 cm/sec. while calculation shows a loss of 23.7 cm/sec. This theoretical calculation gives however a maximum figure. A minimum figure by the same method may be obtained by considering the

[2] For the purposes of these calculations the center of gravity was considered to be 10 cm. above the hip joints. If this distance is taken as 20 cm. the angle of contact is only increased 2° and the average forward pressure component is reduced from 23.7 to 20.4 cm/sec.

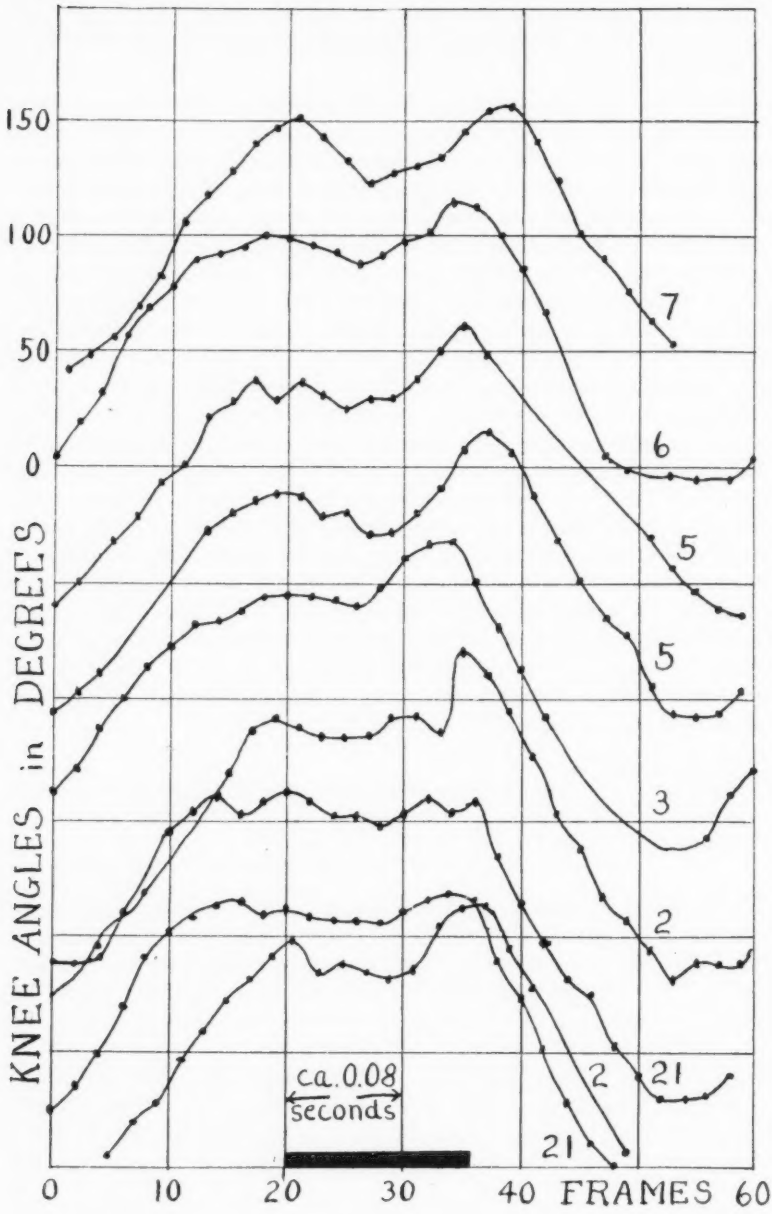


Fig. 16. Changes in the angle of the knee during the running cycle. Each square vertically represents 50 degrees. The figures at the left apply only to the uppermost graph. Each succeeding graph is displaced downward 50 degrees from the one above with the exception of the lowermost one which is plotted on the same ordinates as the one above it.

runner as falling for only half the period between contacts in which case his vertical velocity at the moment of contact is half as great and the forward pressure component of his velocity is almost exactly half as great or 11.9 cm/sec. which is in striking agreement with the value of 13 cm/sec. obtained from the platform.

In objection to this theoretical calculation it might be pointed out that the knee bends at the moment of contact in such a way that the impact of the body does not develop all at once but is distributed over the next few hundredths of a second following the contact. During this time the center of gravity will move forward until it is directly above the point

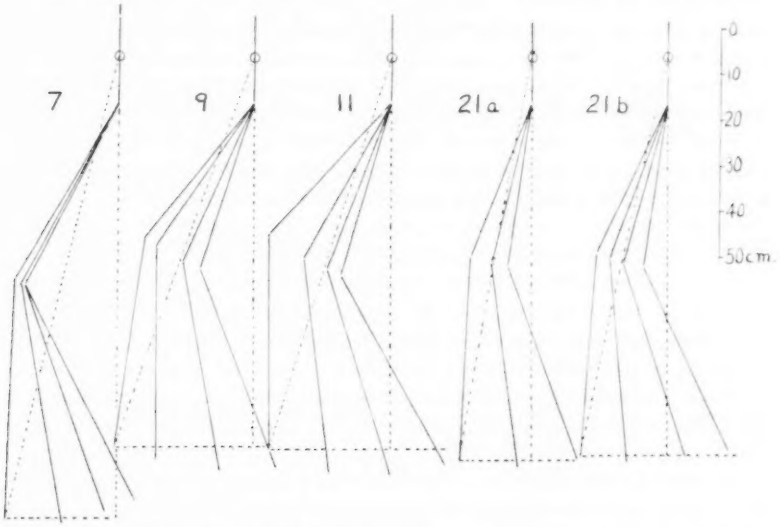


Fig. 17. Positions of the leg drawn to scale at the moment of contact with the ground and at intervals of two frames thereafter. The dotted lines from the center of gravity of the runner to the point of contact measure the angle of contact.

of contact when no forward pressure can be exerted. It will be shown below that this objection is not well founded. Meanwhile in order to be clear concerning exactly what does happen at the moment of contact, figure 16 has been prepared in which the angle made at the knee between the upper and lower legs has been calculated and plotted. These curves are all oriented in respect to time so that the moment of contact for each curve coincides with the point indicated on the contact block at the bottom of the diagram. It may be seen that in every case there is a more or less obvious *decrease in the angle of the knee immediately after the moment of contact.*

Since the durations of foot contact differ in different runners it is impossible to make the points of breaking contact coincide exactly but the deviation from the point marked is not great. It is evident that at the point when the toe leaves the ground there is a more or less marked increase in the knee angle, after which the knee is rapidly flexed against the buttocks.

Figure 17 is drawn to illustrate the rather surprising fact that in spite of this slight bending of the knee the body is not thereby lowered. Because of the forward motion of the body the extension of the thigh more than compensates for the flexion of the knee. Data from five different contacts are included in figure 17. The figure represents to scale the positions of the leg at every other frame following the moment of contact. The first position on the left represents the position at the first exposure after contact was made. The others follow at intervals of 0.016 second (0.0136 sec. for no. 21). The diagonal dotted line connecting the center of gravity with the point of contact determines the angle of contact. The center of gravity is taken as located 10 cm. above the hip-joint. The main point to be observed is that the vertical distance between the center of gravity and the point of contact tends to increase. The amount of this increase is only 1 to 2 cm. in runners 7 and 21, but is 5.5 cm. in nos. 9 and 11. It would appear therefore that the "ground component" (fig. 15) of the impact of the body with the ground is transmitted to the ground without appreciable loss due to bending of the knee for there is no apparent "give" in the leg in the direction of this component.

It can further be seen from figure 17 that the point of contact is ahead of the center of gravity for about 0.03 second after the moment of contact and this agrees reasonably well with the duration of the forward pressure as recorded on the platform. From the above discussion it is clear that the foot could exert forward pressure upon the ground even though the foot itself were not moving forward at the moment of contact as was suggested in connection with the data of figure 14.

An interesting point of minor importance comes out of a comparison of figures 4 and 15. The latter shows that the hips tend to rise from 1 to 5 cm. immediately after the foot makes contact with the ground. The former shows that the height of the ear or the neck diminishes in most cases 1 or 2 cm. immediately after contact. This diminution can be discovered by observing the fall of the upper curves (solid lines, fig. 4) immediately after the point of contact which is indicated on each curve, by a small arrow. It appears difficult to reconcile these two findings, the simultaneous rise of the hip joint and fall of the ear or neck, unless the body is compressed slightly by the impact with the ground, possibly by a slight bending of the vertebral column, or tilting of the pelvis.

Discussion. Reference may be made to the theory of Graham Brown

(1912) that the movements of a runner are analogous to the undulating movements of birds in flight. Mechanically this means that the runner bounces off the ground. This question reduces itself to the same question which was discussed previously (Fenn, 1930) in connection with the swinging movements of the limbs. When the forward movement of a limb is checked is its potential energy thereby stored for the back swing? Likewise, does the downward impact of the body store energy in the extensor muscles of the leg which can help to raise the body at the next take-off? At the moment of contact both the thigh and the knee are being extended. After the contact the thigh continues to extend but the knee flexes slightly as the body rides up on the leg, on a small scale somewhat as a pole-vaulter rides up on his pole. The knee is thus flexed 10° more or less by the impact and then continues to extend at the take-off. A bounce implies storage of energy and the only place where such storage seems possible is in the forcible stretching of the knee extensors. They are put into a position where they can develop a *passive tension* which according to Bethe (1919) exceeds their *active tension*. This passive tension must be maintained by a continuous expenditure of energy and it would seem that all the body could have gained was the energy which would otherwise have been necessary to develop this tension. It sould seem from the point of view of the energy changes that a runner bounces to a very limited extent.

Some attempts were made to discover from the film some differences in form in different runners to explain why some were fast and others slow. The fastest runner studied, no. 21, with a velocity of 8.2 to 8.45, 8.5 meters per second,[3] was outstanding among 32 others examined chiefly in his length of stride which was 191 to 194 cm. long. Only five others had a step over 190 cm. long, and only seven others had a step 185 cm. or over. No. 21 took 4.35 steps per second; only 6 others attained this frequency. Only 9 of the others had a frequency below 4 steps per second. The only difference in form which could be established between no. 21 and the other 32 runners was in the height to which the leg was raised in front of the body. In three different runs no. 21 raised his thigh to a maximum angle of 7, 9, and 9 degrees respectively with the horizontal. Four other runners reached angles of 14 to 16 degrees, 4 reached angles between 18 and 22 degrees, 7 reached angles between 23 and 26 degrees. The legs of all the others were still lower.

One further suggestion concerning a detail of good form in running is suggested by figure 17 where it is seen that the fast runner, no. 21, establishes contact with the ground at a larger angle (with the horizontal) than nos. 9 and 11. Presumably he wastes less energy in forward pressure on the ground (cf. also Jokl, 1929).

[3] The world's record for the 100 yard dash is 9¾ seconds. Assuming 1 second lost in starting this gives a maximum speed of 10.63 meters/sec. which is much larger than that here recorded.

It has been shown that the center of gravity rises 6 cm. off the ground at each step in running. It has also been shown that this rise is not necessary in order to keep the body in the air the observed length of time. Why then is this part of the external work of running necessary? It must be regarded doubtless as a compromise between the varying demands upon the body in running. Part of this rise is due to the flexion of the knee as the leg is brought forward. This serves to diminish the moment of inertia of the leg so that less force is required to carry it forward at the requisite angular velocity. The arms also contribute somewhat to this 6 cm. rise of the center of gravity but they presumably do this, in spite of the trifling energy loss, in order to compensate for the twist given to the body by the opposite movements of the legs. The remainder of this 6 cm. rise seems to be a measure of the length of time during which the body is falling freely. This is presumably short in smooth runners and long in poor runners who appear to run "like sewing machines." The period of fall could be much shortened by giving the body an upward impulse on taking off. If the body were going up during half the period of suspension then the distance of fall would be only one-fourth as great.

Conclusion. In running there are therefore vertical movements of the body and changes in horizontal velocity which account for an expenditure of energy of 0.6 to 0.7 horse power. By itself this is a small item but not negligible. Changes in the kinetic energy of the limbs account however (Fenn, 1930) for a further 1.7 horse power so that the total measured is 2.3 horse power. This does not account for the total working power of the sprint which was estimated from the acceleration of the runner, by Furusawa, Hill and Parkinson (1927) as $8\frac{1}{2}$ horse power at the maximum and by Gertz (1929) as 6 to $8\frac{1}{2}$ horse power. It is, however, an appreciable fraction of the total energy of the sprint which we have determined from the total oxygen consumption as 13 to 15 horse power. The mechanical energy expenditure is a still larger fraction of the total if the work of fixating the joints and the trunk and the work of antagonistic muscles (if any) be included also. The fraction of the "initial" energy left for Hill's (1927) "viscosity factor" is then not as great as was at first supposed. One wonders therefore whether Hill's analogy between a runner and a falling drop is not more mathematical than real. Is the resisting force which appears to increase in proportion to the velocity of the runner a real "viscosity factor" or merely a conglomeration of opposing influences, depending for example on the way in which the runner changes his stride with increasing velocity? To answer these questions further detailed study is necessary.

## SUMMARY

1. From measurements made on moving picture films of sprinters, figures are obtained from which some of the work done in running can be calculated.

2. The movements of the center of gravity within the body due to changes in the positions of the limbs are determined. It tends to move first upwards and backwards, then downwards and forwards twice in each running cycle (fig. 8).

3. Both arms and legs tend to raise the center of gravity within the body at the end of the period when the foot is in contact with the ground, the total average rise being 2.9 cm. Simultaneously the body itself rises an average distance of 3.1 cm. ,the total rise of the center of gravity in relation to the ground being therefore 6 cm. This rise occurs while the foot is on the ground. The corresponding rate of energy expenditure is 0.215 horse power (fig. 4).

4. Backward and forward movements of the limbs are largely compensatory on the two sides of the body so that the horizontal movements of the center of gravity within the body are reduced to small dimensions (figs. 5–7).

5. The velocity of the head is greater than that of the pelvis while both feet are in the air but the reverse is true while either foot is in contact with the ground (fig. 9).

6. The changes in velocity of the pelvis are not as great as they appear to be from direct measurement of the film because there is a compensatory movement of the center of gravity within the body which is shown to move backwards when the apparent forward velocity of the pelvis is at a maximum (fig. 10).

7. An apparatus (fig. 11) is described by which the horizontal pressure of the foot on the ground is measured during sprinting. During each period of contact there is first a forward pressure and then a backward pressure (fig. 12). This constitutes a direct method of measuring energy loss due to fluctuations in the forward velocity.

8. The forward pressure on the ground represents for the whole run a loss of 0.34 horse power and the backward pressure an expenditure of 0.5 horse power (fig. 13).

9. The difference between these two values is 0.16 horse power which is due to wind resistances and agrees with independent estimates of this quantity (table 1 ) by duBois-Reymond and A. V. Hill.

10. At the moment of contact with the ground the foot is stationary or is moving slightly forward in relation to the ground but backwards in relation to the body (fig. 14).

11. The knee is bent slightly immediately after the foot touches the ground and is suddenly extended when the foot leaves the ground (fig. 16). The loss of velocity due to contact of the foot with the ground is 13 cm/sec. as measured on the special apparatus (running platform) described and it is 12 to 24 cm/sec. when calculated from the vector velocity of the center of gravity of the runner and the angle of contact with the ground (fig. 15).

13. The positions of the legs are such that the level of the hips must rise more or less immediately after the foot touches the ground (fig. 17) while measurements of the films indicate a slight fall of the level of the ear at this time.

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# STUDIES IN VOLUME CHANGES OF THE PANCREAS

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During the last several years a technic has been developed whereby an abdominal viscus may be enclosed in a plethysmograph where it heals in position thus permitting a study of volume changes over a long period of time. Volume changes in the spleen (Hargis, 1926), liver (Mattson, 1929), and kidney (Reid, 1929) have been studied. It was desired to make a similar study of the pancreas with particular regard to whether this organ responded to reflex stimulation and to substances which have to do with carbohydrate metabolism. However, the technic was not found suitable for prolonged observation on pancreatic volume, because as soon as a portion of the pancreas was isolated and enclosed in a plethysmograph it underwent rapid degenerative changes making physiologic observations impossible. Accordingly a study of the volume changes of the pancreas following the injection of various drugs and various procedures was made in anesthetized animals.

A few studies on this problem have been made. Francois-Franck and Hallion (1896, 1897) obtained vasoconstriction and a decrease in pancreatic volume following the stimulation of the sympathetic nerves to the organ, and an increase in pancreatic volume following the stimulation of the peripheral end of the vagus. May (1904) found a decrease in pancreatic volume following stimulation of the vagus as well as of the sympathetic fibers, and following the intravenous injection of epinephrine. Edmonds (1911) and Mann and McLachlin (1917) obtained a decrease in pancreatic volume following the injection of adrenalin.

METHOD OF EXPERIMENTATION. Etherized dogs were used. Plethysmographs made from gauze and non-flexible collodion suitably modified to fit the pancreas, as well as the ordinary type of glass plethysmograph were employed. As much of the pancreas as could be obtained without gross disturbance of the blood vessels and possibly nerves was enclosed in the plethysmograph in the usual manner. The plethysmograph was connected to the modified piston recorder of Lombard and Pillsbury, described by Mason, by means of a rubber tube through a suitably placed stab incision.

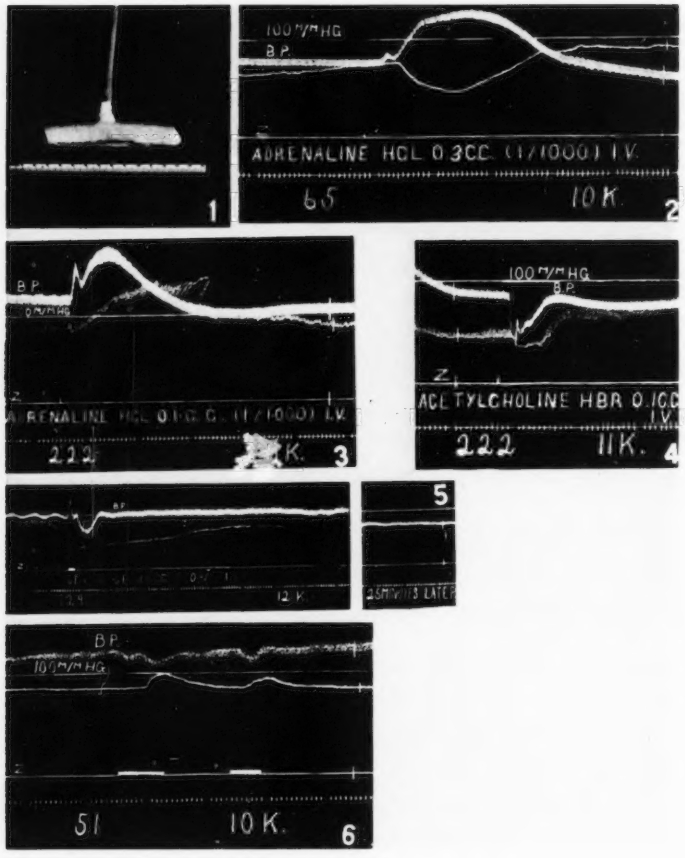


Fig. 1. Pancreatic plethysmograph.

Fig. 2. Decrease in pancreatic volume following intravenous injection of epinephrine.

Fig. 3. Increase in pancreatic volume following intravenous injection of epinephrine.

Fig. 4. Effect of acetylcholine on pancreatic volume; acetylcholine solution is in the dilution of 1 mgm. in 1 cc.

Fig. 5. Tracing showing the rise in pancreatic volume following the intravenous injection of glucose.

Fig. 6. Effect of compression of dog's testes (dog under surgical anesthesia).

RESULTS. All drugs were given intravenously. Epinephrine 1:1,000 given in doses of 0.1, 0.2, and 1 cc. gave reaction of either a decrease or an increase in pancreatic volume, the result not being predictable. When the reaction was manifested by a decrease in volume, there was occasionally an initial increase, and when it was manifested by an increase, the increase was abrupt. The return to normal in both cases varied; it usually coincided with the return of the blood pressure to normal, but occasionally it was delayed (figs. 2 and 3).

Histamine given in doses of 0.2, 0.3, 0.4, and 0.5 mgm. similarly caused reaction of either an increase or a decrease in pancreatic volume. The increase as well as the decrease was abrupt, and the return to normal was more gradual. The decrease in volume was obtained only in vagotomized dogs.

Nitroglycerine was given in doses of 1.3 mgm. Here again a reaction resulted in either an abrupt increase or a decrease in pancreatic volume.

Acetylcholine gave two reactions, depending on whether the animal was atropinized. If the animal was atropinized the well known vasodilatation was manifested by a fall in blood pressure and a rise in pancreatic volume. If he was not atropinized, there was the expected fall in volume which accompanied the profound vagal inhibition of the heart (fig. 4).

Pilocarpine was given in doses of 6.5, 13 and 19.5 mgm. The usual reaction was that of an abrupt decrease in pancreatic volume followed by a minor increase in volume. The blood pressure showed an initial fall followed by a sharp rise which was possibly of asphyxial origin. The changes in volume were apparently secondary to the blood pressure.

The reaction to pituitrin consisted in a decrease in pancreatic volume. The drug was given in doses of 0.5 cc. Subsequent injections gave only slight decreases, or failed to produce any change either in volume or blood pressure.

Atropine sulphate was given in doses of 1.3 mgm. The reaction was a sharp rise in volume which was sustained for varying periods of time. The return to normal was more gradual. The blood pressure showed an abrupt rise followed by an abrupt fall below normal, which was also sustained for varying periods of time. The pancreatic volume and blood pressure returned to normal about the same time.

Sodium chloride (12 per cent), in amounts of 10, 20, 30, and 40 cc., provoked an increase in pancreatic volume which was sustained. The return to normal was very gradual. The blood pressure showed a similar curve. Similar results were obtained with 5 cc. of 16 per cent solution.

Dextrose solution (50 per cent) given in amounts of 10, 20, 30, and 40 cc. produced results similar to those of sodium chloride with the exception that the rise after dextrose was sustained for a longer period of time. Five cubic centimeters of 75 per cent solution gave similar results (fig. 5).

Sodium nitrite was given in doses of 16.2, 48.6, and 64.8 mgm. An increase in pancreatic volume resulted. The blood pressure fell and returned gradually to normal.

Witte's peptone (3 per cent) in doses of 30 cc. provoked an increase in volume, and a decrease in blood pressure. The return to normal of both volume and blood pressure was gradual.

Compression of the dog's testes with the dog under a surgical anesthetic provoked an increase in pancreatic volume accompanied by a slight fall in blood pressure (fig. 6).

Distilled water in doses of 10 cc. did not cause a change in pancreatic volume.

Ninety-five per cent alcohol given in doses of 0.5 cc. in 2 cc. of distilled water, and 4 cc. in 4 cc. of distilled water gave a slight decrease in pancreatic volume with an accompanying fall in blood pressure.

Ether, 1 to 3 cc. dissolved in physiologic sodium chloride solution, provoked first an initial increase and then a slight decrease in pancreatic volume, followed by an increase. The return to normal was gradual. The blood pressure fell sharply, and returned to normal gradually.

Rattlesnake venom, 0.3 cc. of 2 per cent solution and 0.4 cc. of 4 per cent solution, was given. The pancreatic volume and the blood pressure both fell sharply, and were sustained for a considerable time. Later there was a fluctuating rise in pancreatic volume.

COMMENT. There are reasons for believing that following the intravenous injection of glucose the pancreas is provoked to secrete insulin. Thus German and Barr (1927) showed that marked alterations in the electric potential of the pancreas developed after glucose was injected. In view of the fact that an organ which is active has ususlly an increased blood supply it was hoped that the intravenous injection of glucose would provoke a distinct rise in volume greater than could be accounted for on the basis of the osmotic pressure of the injected glucose. Although the glucose injected provoked a considerable rise in pancreatic volume, it was not decisively greater than an osmotically equivalent quantity of sodium chloride solution. It is noteworthy in this connection that Anrep did not observe rise in pancreatic volume following the injection of depressor-free secretin.

The response of the pancreas to rattlesnake venom is of interest in that its behavior is identical with that of the liver, spleen, kidney, and intestine, as reported by Essex and Markowitz. It appears that all the splanchnic viscera undergo a decrease in volume immediately after the injection of rattlesnake venom.

The twofold reaction of the pancreas to epinephrine and to histamine is difficult to explain. Epinephrine and histamine each act to constrict arterioles and dilate capillaries. The effect on pancreatic volume might depend on which one of these two factors is greater at the time of injection.

Another possibility in case of a rise in volume after the injection of epinephrine is that the blood becomes crowded into this organ. One might recall in this connection that the normal response of skeletal muscle to epinephrine is a rise in volume. Otherwise the volume changes of the pancreas have not been exceptional.

Successive injections of pituitrin were followed eventually by failure of the pancreas to respond to the drug, which result is comparable to the effect of this drug on blood pressure.

My failure to apply a plethysmograph to the pancreas so that records of pancreatic volume could be taken over long periods of time may be compared with the similar inability of Mason to obtain prolonged plethysmographic records of the thyroid gland. The cause of this failure, however, is different. In the case of the thyroid gland the absence of a structure like the great omentum that would throw out a deposit of inflammatory lymph is an impossible handicap. Although this factor is not operative in the case of the pancreas, an equally serious difficulty is encountered in the marked tendency of the traumatized pancreas to undergo autodigestion.

## SUMMARY

A method is described of making a collodion plethysmograph for the pancreas which serves admirably for acute experiments. The changes in pancreatic volume thus obtained are reported for a number of drugs and procedures. The results in general are in harmony with the normal vascular responses to these drugs and procedures.

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# GALVANIC SKIN REFLEX FROM ANIMALS WITH COMPLETE TRANSECTION OF THE SPINAL CORD

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The experiments described in this paper are concerned with the question as to what parts of the central nervous system are necessary for the production of the galvanic skin reflex. Previous workers have found that this phenomenon is not affected by removal of the cerebral hemispheres (Schilf and Schuberth, 1922), by section through the mid-brain (Fauville, 1921), by removal of the mid-brain and cerebellum (Foá and Peserico, 1923), by section through the brain-stem at the isthmus (Schilf and Schuberth, 1922; Sakamoto, 1924). A section through the hind-brain segments, however, causes the reflex to disappear. Schilf and Schuberth drew the conclusion that the cells upon which the reflex depends must be located in the region of the isthmus or in the upper part of the medulla oblongata and not in lower parts of the central nervous system. That is, they believe that there is a center in the brain-stem for the galvanic reflex similar to the respiratory center. This conclusion was rather unexpected in view of our definite knowledge of the close relation between the galvanic reflex and the sweat-glands and of the presence of autonomous activity of sweat-mechanisms in the spinal cord. Thus the observations made by Head and Riddock (1920) on spinal man demonstrated beyond doubt that spinal sweat-mechanisms are capable of a high degree of activity, independent of influences from higher centers. It was shown that excessive sweating of the body below the level of the transection could be produced reflexly by any mild form of stimulation, such as stroking of the sole of the foot. And in keeping with this observation, Wang, Pan and Lu (1929) found that the galvanic skin reflex could be elicited in acute preparations of spinal animals by electrical stimulation of afferent nerves. In the present experiments it was found that in chronic preparations of spinal cats the galvanic skin reflex can be obtained by any form of tactual stimulation and that it is more active than in normal animals. In other words, it is now well established that the system necessary for the production of the reflex is located in the cord rather than in any supra-segmental or cranial parts of the central nervous system.

METHODS. The technique used was essentially the same as that de-

scribed in previous experiments on the skin resistance changes produced in cats by total nerve section and by sympathectomy. (Richter, 1929.)

The reflex was registered photographically by means of a string galvanometer and a camera. The electrodes consisted of small pieces of zinc sheeting and a paste made of kaolin and saturated zinc sulphate solution. The paste served to make an intimate contact between the pieces of zinc and the pad of the foot. Only the large central pad was used and it was separated from the rest of the foot by means of a narrow strip of rubber sheeting in which a hole had been cut. After the pad was pushed through the hole the rubber strip was tied firmly around the foot. Then the electrode was adjusted to the pad and was held in place by another rubber strip tied around the foot. One electrode was attached in this way to each of the four feet.

The E.M.F. or electro-motive-force method of obtaining the galvanic reflex was used in these experiments, that is, no external current was employed. When an external current is used this phenomenon manifests itself in a reflex decrease in the electrical resistance of the skin. In the present experiments the records show the changes in potential between the two electrodes. It is a well-known fact that when using this E.M.F. method the electrodes must be attached, one to an area rich in sweat-glands, the other to an area in which the sweat-glands are relatively scarce. In man usually the palm and back of the hand are used. No effect is obtained when both the electrodes are attached to the palms because the effects are opposite and equal, and cancel one another. In the cat the second electrode cannot be attached to the back of the foot because of the presence there of a thick, coarse hair and the consequent difficulty of obtaining a good contact with the skin. For this reason the second electrode is attached to the inner surface of the pinna of the ear, which is usually almost entirely free from hair. The electrode is fastened to the ear by means of a small bull-dog clamp.

Because of the fact that the ear does not produce any galvanic changes, it was possible in this way to obtain monophasic galvanic changes, that is, only the changes occurring on the pad of the foot.

The spinal cord was completely transected between the 1st and 2nd thoracic roots in fifteen cats and the operation was checked by autopsy. The transection was made at this level in order to interrupt the sympathetic impulses from higher centers to the fore-limbs as well as to the hind-limbs. The sympathetic rami to the fore-limbs leave the cord beginning with the 3rd and 4th thoracic nerves. Records were taken in all of these experiments of the galvanic skin reflex from the fore-paws as well as from the hind-paws.

For the purpose of the experiment, as will be seen below, it was necessary to keep the animals alive as long as possible after the transection. Because

of the temporary inability of the animals to maintain their normal body-temperature they were kept warm with electric pads. They were fed until they were able again to take food by themselves, and for reasons of cleanliness they were kept in hammocks. In this way some of the animals were kept alive for six months or longer.

RESULTS. A typical record of the galvanic skin reflex from a spinal cat is shown in figure 1a. The time is indicated in seconds at the top of the record, and the stimulus at the bottom. The electrodes were attached to the left hind-foot and to the pinna of one ear. The stimulus used was a pin-prick applied to the tail. In this cat the spinal cord was completely transected between the 1st and 2nd thoracic nerves twelve weeks before. Similar records were obtained from the fourteen other cats with the same lesion.

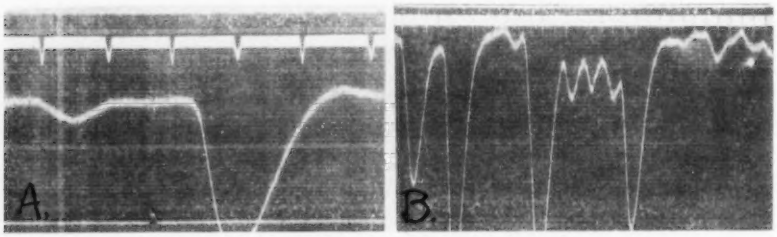


Fig. 1 a. Record of galvanic skin reflex produced on the left hind-foot of a spinal cat ($T_1$–$T_2$) by pinching the tail. Time is given in seconds at the top of the record, and the incidence of the stimulus at the bottom.

Fig. 1 b. Record showing spontaneous galvanic fluctuations in a spinal cat ($T_1$–$T_2$). The animal was perfectly quiet during the time this record was taken. Time is given in seconds.

The stimuli necessary to elicit the reflex were naturally limited to those which could be brought into direct contact with the skin, all other sensory channels to the spinal cord besides the tactual having been cut off. Pin-pricks, pinches, electrical shocks applied to any point below the level of the lesion were sufficient to elicit the reflex. The tail, especially the region near the base, produced the largest responses both in the fore and hind-paws.

The general type of the response is much the same as is obtained from direct electrical stimulation of the sympathetic chain with make and break or interrupted shocks. Only monophasic negative deflections were obtained, just as from make and break shocks of the sympathetic chain. The latency period averaged 0.63 second, which is about the same as that found when the chain was stimulated. The amplitude also was usually rather great, as great as is obtained in very responsive normal cats or when a chain

is stimulated with a maximal current. The duration of the response was about the same as that of the direct response. In some instances it was less than two seconds. It may be noted that this is shorter than the response obtained from a normal intact cat, which is usually at least five seconds.

Apart from these reflex responses the spinal cat shows numerous spontaneous galvanic fluctuations very much the same as are found in frightened normal cats. A typical record of such spontaneous fluctuations is shown in figure 1 b. That they are found in spinal cats was an unexpected observation, for in previous experiments the results had indicated that these waves have their origin in the brain-stem or in higher centers of the brain. These experiments show that they have their origin in the spinal cord.

The appearance of the galvanic reflex is definitely dependent on the degree of the spinal shock and to the extent of the recovery from the shock. For this reason the reflex is rarely found before the tenth day after the transection and in some instances not for twenty or thirty days or even longer periods. Its appearance always follows rather than precedes the return of the motor reflexes. During the period of shock the reflex cannot be elicited by any stimulus however strong, but after the cord has recovered fully, that is, when the motor reflexes are hyper-active, the bladder and rectum are functioning regularly, the galvanic reflex also is hyper-active. It can be elicited from even the slightest touch applied to the tail or any other part of the skin below the level of the lesion. At the same time the spontaneous waves, which also are absent during the period of shock, become very prominent.

This period of increased activity of the galvanic reflex and of the spontaneous waves in the spinal cats undoubtedly corresponds to the period of excessive sweating described by Head and Riddock in spinal man. In the cats this period of hyper-activity continued for months, in all cases up to the time the animals were sacrificed, which in one animal was eight months after the transection.

In so far as the dependence of the galvanic skin reflex on the sympathetic nervous system is concerned, it is interesting to note that during the period of shock when the reflexes and spontaneous waves were entirely absent on the hind-feet, both were absent on the fore-feet as well, even though the motor activity of the fore-feet was perfectly normal. This observation demonstrated clearly the dependency of the galvanic skin reflex on the sympathetic cells of the spinal cord in confirmation of similar observations made by Schilf and Schuberth, and Dennig (1924) and others.

It is established then that the centers necessary for the production of the galvanic reflex are located in the segmental levels and not in higher levels of the brain-stem and brain. This does not mean, however, that in the intact animal the cells higher up in the cord and brain-stem and hemispheres may not play a rôle in these responses. It was shown (Wang and

Richter, 1928) that electrical stimulation of the tuber cinereum produces a typical galvanic reflex on any of the feet.

The galvanic skin reflex should be of value in the study of spinal vegetative reflex activity, and of the localization of the sympathetic centers and pathways in the spinal cord.

## SUMMARY

1. After a period of "shock" following a complete transection of the spinal cord of cats at $T_1$ or $T_2$, the detached segments show a galvanic reflex. The response can be elicited by tactual stimulation, pinch, pinprick, electrical shock applied at any point below the level of the lesion, and it is present on all four feet regardless of the location of the stimulus.

2. These experiments demonstrate clearly that the segmental and intersegmental mechanisms are sufficient for the production of the reflex, independent of the hind-brain, mid-brain or thalamus.

3. The type of the response is essentially the same as that obtained from electrical make and break shocks applied directly to the sympathetic chain. Only monophasic negative waves were obtained; the latency period was 0.63 second, just slightly longer than that from direct sympathetic stimulation. The amplitude was the same as is obtained by maximal sympathetic stimulation; the duration averaged 2.5 seconds, which is rather short as compared to the response from an intact animal, but the same as is produced by single break shock stimulation of the sympathetic chain.

4. Besides the reflex galvanic response the spinal cats show spontaneous galvanic deflections much the same as are obtained in normal intact cats, which are in a frightened condition.

5. The appearance of the reflex and of the spontaneous waves is dependent on the degree of spinal shock produced by the transection. The appearance of the reflex always follows and never precedes the appearance of the motor reflexes. It usually does not appear for eight or ten days, sometimes not for twenty to thirty days.

6. Several weeks after the transection when both motor and galvanic reflexes are fully restored, the galvanic reflexes become so hyper-active that they can be elicited often by only a slight touch, and the spontaneous waves become very prominent also.

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# NEURAL AND HORMONAL INFLUENCES ON BODILY ACTIVITY

## Differential Factors Controlling the Heart Rate During Emotional Excitement

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Within recent years increasing importance has been referred to the frequency of the heart beat in the evaluation of bodily functions. After reviewing a large body of evidence Henderson (1923) concluded that the factors regulating the heart rate, along with the venopressor mechanism, served to adjust the circulation rate to the energy expenditures and respiratory metabolism of the body during normal activity. The stroke volume of the heart in rest and in work, in man and other mammals of all sizes, he considered as a nearly constant quantity for each individual (1.5 to 2.0 cc. per kilo body weight). Further studies have led Henderson and his associates (1927) to maintain that in man the circulation varies in proportion to the pulse changes, and that this is the normal relation. Different conditions are said to be present in the case of the athlete's heart, which is regarded as supernormal. Grollman (1929) has shown that in psychic disturbances in man there occur concomitantly with cardiac acceleration noteworthy increases in the heart output as well as in arterial pressure. Under certain anesthetics, changes in pulse rate are accompanied by similar changes in cardiac output (Tappan and Torrey, 1926), although in unanesthetized dogs this relationship has been found by Marshall (1926) usually to be absent. In experiments on shivering during exposure to cold and also in pregnancy it was observed by Marshall, however, that increases in heart rate accompanied increments in minute volume.

That alterations in venous pressure and in heart rate during exercise follow similar trends has been indicated by many workers (White, 1924; McCrea, Eyster and Meek, 1928). Definite correlations between the pulse and cardiac and general metabolism have also been established (Evans 1912; Benedict, 1915; Smith, 1922). Further considerations on the heart as a representative organ capable of yielding significant data concerning body processes have been discussed elsewhere (Cannon, Lewis and Britton, 1926). More intimate knowledge of the extent of the control exercised by various factors on cardiac activity would therefore seem to be desirable.

A number of agencies are known to affect the frequency of the heart beat. Beside nervous influences and thermal and ionic changes in the blood stream, other less well understood factors may bring about modifications in cardiac responses. A material which is discharged from the liver, and also metabolites given off by contracting muscles, have under certain conditions been observed to accelerate the pulse (Cannon *et al.*, 1921, 1924). Recent evidence (Cannon and Britton, 1925, 1927) offers proof of notable effects on the central circulatory mechanism of secretion from the adrenal medulla during emotional reactions. Among the foregoing it appears that nervous (vagus and sympathetic) and humoral (chiefly adrenal) influences only are able to evoke significant *quick* changes, i.e., those occurring immediately or within a few minutes after excitation, of the normal heart beat. Those changes which may be mediated by hepatic substances are negligible in the fasting animal, and thyroid secretion apparently produces only slowly-rising variations in heart rate (unpublished experiments).

In a previous paper in this series (Britton, 1928) the factors concerned in the release of glucose from the liver into the blood stream under stressful demands were analysed. In the experiments considered here it was sought to differentiate the respective parts played by the vagus nerves, the thoracic sympathetic (accelerator) nerves, and the secretion of the adrenal medulla, representing the three important factors concerned, in the regulation of the heart rate during normally aroused emotional activities. Harrison and his associates concluded (1927), it may be said, that the cardiac nerves exert a marked influence on the pulse and practically no influence on the minute output of the heart, while the adrenal hormone causes changes of intermediate degree in both pulse rate and minute output.

The fundamental and instinctive reaction which is offered by the cat in the presence of a dog was used in this study as typifying a very common physiological response. Such a reaction is usually attended by moderate and sometimes vigorous muscular activity. In the present experiments the animals employed—cats selected for their readiness of response—were somewhat restrained physically by confinement in a small cage during the excitation period, as previously described (Britton, 1928). In all cases graphic records of the basal heart rate, with the cat quiet and resting on the lap, were first taken with a double-tambour recording-system (Cannon, Lewis and Britton, 1926); the animal was then caged and subjected to the sallies of a barking, aggressive dog for one and a half minutes, after which further cardiac records were secured. With practice the heart-beat could be recorded within 5 to 15 seconds after the excitement period. To avoid the effects of body temperature and other changes which might vitiate the results, only those variations which were observed within a minute or so following the emotional reaction are considered in this report.

The responses were determined *a*, in normal animals; in animals *b*, with one of the three cardiac regulatory (and acceleratory) factors eliminated; *c*, with two of the factors removed, and *d*, with all three factors removed. Different combinations were tested under *c* as indicated later. The sympathetic factors were removed by excision of the stellate ganglia,

TABLE 1

| BEFORE OPERATION | | | EXPERIMENTAL CONDITIONS | AFTER OPERATION | | |
|---|---|---|---|---|---|---|
| Cat number | Number of times excited | Range (and average*) of increase in heart rate | | Cat number | Number of times excited | Range (and average*) of increase in heart rate |
| | | | (A) | | | |
| 152 | 2 | 34–44 (39) | Vagus nerves only removed | 152 | 3 | 16–60 (40) |
| 154 | 2 | 56–68 (62) | | 154 | 4 | 24–60 (44) |
| 153 | 3 | 20–56 (43) | Sympathetics (accelerators) removed | 153† | 2 | 18–36 (27) |
| 172 | 3 | 30–70 (51) | | 172 | 4 | 30–72 (52) |
| 156 | 2 | 36–48 (42) | Adrenal medulla removed | 156 | 4 | 28–64 (51) |
| 157 | 2 | 48–72 (60) | | 157 | 4 | 32–52 (40) |
| | | | (B) | | | |
| 156 | 2 | 36–48 (42) | Sympathetic and medullo-adrenal factors removed (vagus nerves intact) | 156 | 12 | 12–32 (16) |
| 157 | 2 | 48–72 (60) | | 157 | 3 | 8–32 (21) |
| 158 | 2 | 44–74 (59) | | 158 | 15 | 20–56 (38) |
| 169 | 3 | 64–80 (70) | | 169 | 7 | 32–56 (40) |
| 152 | 2 | 34–44 (39) | Adrenal medulla and vagi removed (sympathetic nerves intact) | 152 | 11 | −4–36 (17) |
| 154 | 2 | 56–68 (62) | | 154 | 6 | 8–60 (34) |
| 162 | 3 | 36–68 (50) | | 162 | 4 | 12–28 (18) |
| 170 | 3 | 64–80 (69) | | 170 | 10 | 16–64 (34) |
| 155 | 3 | 54–92 (78) | Vagus and sympathetic nerves removed (heart denervated) (adrenal medulla intact) | 155 | 7 | 24–52 (36) |
| 161 | 3 | 60–72 (67) | | 161 | 9 | 20–52 (35) |
| 174 | 3 | 54–78 (68) | | 174 | 3 | 33–54 (46) |

* Given parenthetically for clearness and rough comparison only (see text).
† Slight infection developed.

the thoracic trunks (6 to 8 ganglia), and the mediastinal (sympathetic) fibres; the vagal influences by cutting out 3 to 4 cm. of the right vagus and all the cardiac branches of the left trunk, as far as the esophagus; and the adrenal medulla by curettage and suction, or sometimes by denervation of the glands. In a few cases the liver nerves also were ablated, without noticeable difference in the results. Eight to ten-day intervals were

allowed for recovery. The animals were fasted for 20 hours before excitation.

The main results of the experiments are given in table 1 (A) and (B). Varying degrees of response to excitation, as evidenced by the various cardiac increments, are shown by different animals. Following the experimental procedures as enumerated under (A), table 1, relatively slight changes from the normal reactions are observed to occur: the averages and the ranges of increase are not disturbed to any marked extent; sometimes (152, 156) even greater accelerations were noted after operation. It appears therefore that all the vagus fibres to the heart, or the sympathetic (accelerator) nerves, or the medulliadrenal tissues—that is to say, any *one* (group) factor which normally contributes reflexly towards the regulatory and acceleratory influences on the heart rate—may be eliminated without significant effect on the normal pulse changes during emotional and moderate muscular activities. That compensatory hyperfunction of the remaining factors may be called into play in such circumstances is of course possible.

The results given in part (B) of table 1 present a different picture. In some cases the same animals were used as in (A), and the operative procedures were carried a step farther—a second important factor in cardioregulation was removed. It appears clear that the competency of the heart is considerably diminished by such removal, whether a one-stage or two-stage operation be carried out. The elimination of any *two* of the three fundamental mechanisms involved in control of the heart's activity reduces its acceleratory ability, indeed, by approximately one-half that observed in normal animals. In other control operations, it should be said, there occurred no such reduction in activity. The disability persisted after several weeks of experimentation, and as will be shown it cannot be considered due to any tolerance towards the excitatory agent.

In several cases the animals were further tested after completing the cardiac denervation and ablation of the adrenal medulla. Results which have been previously noted (Cannon, Lewis and Britton, 1926) were now reproduced—the heart rate changes after vigorous excitation of the animal were practically negligible. In some instances decreases or no change occurred, and in 18 cases the average increase was 12 beats.

The possibility that animals might become accustomed to frequent excitation, so that the emotional disturbance and the pulse increments might disappear with the passing of time, has been considered (table 2). Three animals were excited for similar (one and a half minute) periods every second day for eight weeks. No diminution in the cardiac reaction appeared at the end of this time. Even a month later one animal (no. 167) showed a marked increase on stimulation. Analysis of table 2 shows indeed that in the last 13 periods of excitement the average increase in two

TABLE 2

| DATE | CAT 165, ♀ | | CAT 166, ♀ | | CAT 167, ♂ | |
|---|---|---|---|---|---|---|
| | Basal heart rate | Increase on excitation | Basal heart rate | Increase on excitation | Basal heart rate | Increase on excitation |
| July 12 | 168 | 56 | 204 | 60 | 192 | 64 |
| July 13 | 220 | 20 | 176 | 92 | 152 | 56 |
| July 15 | — | — | 172 | 44 | — | — |
| July 17 | 196 | 44 | 164 | 64 | 172 | 100 |
| July 19 | 204 | 32 | 176 | 76 | 208 | 28 |
| July 21 | 224 | 40 | 192 | 36 | — | — |
| July 23 | 232 | 24 | 212 | 64 | 204 | 80 |
| July 25 | 176 | 52 | 204 | 64 | 200 | 76 |
| July 27 | 188 | 40 | 168 | 48 | — | — |
| July 29 | 152 | 72 | 160 | 56 | 200 | 56 |
| July 31 | 168 | 72 | 140 | 88 | 148 | 64 |
| August 2 | 164 | 60 | 120 | 64 | 148 | 72 |
| August 4 | 188 | 60 | 168 | 20 | 208 | 44 |
| August 6 | 212 | 64 | 168 | 56 | 164 | 52 |
| August 8 | 200 | 40 | 168 | 48 | 204 | 36 |
| August 10 | 180 | 48 | 160 | 60 | 152 | 52 |
| August 12 | 200 | 76 | 144 | 40 | 136 | 72 |
| August 14 | 200 | 48 | 200 | 58 | 176 | 80 |
| August 16 | 224 | 64 | 200 | 72 | 176 | 74 |
| August 18 | 220 | 20 | 168 | 96 | 180 | 84 |
| August 20 | — | — | 188 | 88 | 176 | 64 |
| August 22 | 204 | 44 | 168 | 68 | 180 | 72 |
| August 24 | 240 | 24 | 176 | 36 | 180 | 92 |
| August 26 | 188 | 64 | 184 | 84 | 188 | 36 |
| August 28 | 212 | 36 | 220 | 64 | 188 | 64 |
| August 30 | 200 | 28 | 212 | 32 | 184 | 56 |
| September 1 | 208 | 48 | — | — | 192 | 44 |
| September 4 | 176 | 44 | 188 | 104 | 188 | 48 |
| September 6 | 208 | 38 | — | — | 192 | 52 |

TABLE 3

| CAT NUMBER | NUMBER OF EXCITATION PERIODS | AVERAGE BASAL HEART RATE | RANGE OF INCREASE ON EXCITEMENT | AVERAGE INCREASE, FIRST 13 PERIODS | AVERAGE INCREASE, LAST 13 PERIODS | AVERAGE INCREASE, ALL PERIODS |
|---|---|---|---|---|---|---|
| 165 | 27 | 205 | 20–76 | 49 | 45 | 47 |
| 166 | 27 | 178 | 20–104 | 59 | 65 | 62 |
| 167 | 26 | 180 | 28–100 | 60 | 64 | 62 |
| All normal cases (25 animals) | 136 | — | 20–104 | — | — | 56 |

instances (cats 166 and 167) was slightly greater than in the first 13 periods, while in one case the reverse condition obtained (see table 3). The likelihood of a fear element entering into the present experiments, in which the animals were restrained in a cage and also subjected to somewhat unusual handling during elicitation of the cardiac record, should be considered in view of the above results.

The rather high basal heart rate shown in the three cases quoted in table 2 may be explained by the fact that young animals were used. It may be noted in this connection that in one animal (no. 165) in which in 16 cases of excitation the average basal rate was at the high level of 213, the average increase was only 39; while in another instance in which the basal rate in 20 cases was 173, the average increase during excitement was elevated to 65 beats per minute. Commonly the basal heart rate varied between 120 and 160. In all the normal cases tested, comprising 25 animals, the range of increase in 136 periods of emotional excitation was from 20 to 104, while the average increase in cardiac frequency was 56 beats per minute.

It has already been shown (Cannon and Britton, 1927) that through medulliadrenal secretion alone, the nervous influences having been completely removed, considerable increments in heart rate may be brought about reflexly during excitement of the unanesthetized animal. The present observation that ablation of the adrenal medulla fails to affect significantly the cardiac reactions if the vagi and sympathetics are intact does not at all invalidate the evidence previously reported. No one factor normally involved in heart regulation appears to possess a prepotent or indeed an indispensable function in this respect. Rather than the usually conceived duality of control by vagal and accelerator factors of the heart rate, a threefold influence through parasympathetic, sympathetic and sympathomimetic mechanisms should be emphasized.

## SUMMARY

Evidence indicating the growing importance which is related to the frequency of the heart beat and its connection with cardiac output and metabolism is briefly discussed. Experimental analysis is made of the various factors involved in regulation of the heart rate in the cat during an intense emotional reaction attended by moderate muscular activity. Quick reflex changes in rate, i.e., those occurring within a minute or so after excitation, are mediated only through vagal, thoracic sympathetic and adrenomedullary influences. Any one of these three influences may be eliminated without significant effect on the normal cardiac increments occurring during excitement.

The removal of *any two* factors normally concerned in regulating the activity of the heart is shown markedly to reduce its acceleratory ability—

usually by about 50 per cent—following vigorous excitation of the unanesthetized animal. This incapacity persists throughout repeated stimulations extending over many weeks. If all three cardio-regulatory factors are eliminated negligible variations only in rate occur during excitement.

In over 100 cases in which normal animals were excited for one and a half minutes the average increase in heart rate was approximately 50 per cent over the basal level. Animals which were subjected to emotional stimulation every second day throughout an eight-weeks' period showed no diminution in cardiac responsé, and thus exhibited no tolerance towards the excitation.

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# THE ABSORPTION AND EXCRETION OF WATER AND SALTS BY MARINE TELEOSTS

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It is a peculiar and interesting fact that marine teleosts excrete urine which is isotonic or hypotonic with respect to the blood, and therefore of considerably lower osmotic pressure than sea water (Rodier, 1899; Bottazzi, 1906; Dekhuyzen, 1905; Burian, 1908, 1910). From this, it appears that these animals are faced with the task of concentrating the surrounding ocean in order to obtain water for the formation of urine, a task which can only be performed at the expenditure of energy in osmotic work.[1] This circumstance might be expected to result in profound differences in the water economy of marine fish as compared with fresh water and terrestial animals to which water is available *ad libitum*.

The answer to the question of what tissue or tissues actually do the work of separating from the sea water the osmotically dilute solution destined to be excreted as urine appears to rest in the determination of whether the renally excreted water is initially absorbed from the intestine or enters the body through the gills. The work to be reported here leads us to the conclusion that water absorption occurs by way of the intestine; but, surprisingly, it is not in the intestine that the osmotic work is done, for the residue formed there as absorption proceeds is actually more dilute than the ingested sea water, and tends to approach the blood in tonicity. This osmotic dilution results from the fact that Na, K and Cl are absorbed from the intestine, along with a considerable fraction of the water, leaving behind only the poorly absorbed Mg and $SO_4$ in highly concentrated state. During this process of concentration some Mg and $SO_4$ are abosrbed, to be subsequently excreted in the urine. The bulk of the Na, K and Cl, together with the bulk of the water absorbed from the intestine, never appear in the urine, however; they are excreted by routes other than the kidneys—

[1] This problem was recognized by Dekhuyzen (1905) who remarks " . . . . la richesse relative en eau de cette urine prove plutôt que les poissons marine resorbent de l'eau, on si l'on prefère une solution diluie effectuant ainsi un travail contre la pression osmotique en consommant de l'energie chimique."

probably by the gills. The salts and water excreted extrarenally, if considered together, would make up a solution hypertonic to sea water and thus compensate for the osmotically dilute urine and intestinal residue.

Thus, extrarenal (branchial?) excretion plays an active rôle in these animals in the regulation of body fluid composition, and relieves the kidneys of much their duty. The consequences of this fact are apparently in evidence in at least one respect: we believe it is the very small volume of urine excreted that is responsible for the absence of glomeruli in the kidneys of many strictly marine fishes.

*Ingestion and absorption of sea water.* That marine fish normally swallow large quantities of sea water is immediately suggested by the fact that there is always present in the intestine proper a considerable quantity of clear and usually colorless fluid. This fluid is frequently discharged from the anus with vigor when the fish is handled, and it can always be expelled into a catheter inserted into the anus by gently massaging the abdomen. Fluid is rarely present in any large quantity in the stomach of starved fish, but this may be due to the rapid passage of ingested water into the intestine where the major process of absorption occurs.

The ingestion of sea water can be readily demonstrated by adding an easily detected substance, such as a dye, to the aquarium water in which the fish are kept. We have found phenol red most satisfactory for this purpose.

In both the sculpin and the eel (in sea water) phenol red can be detected in the fluids removed from the stomach and intestine within 4 to 6 hours after the fish are placed in the tinctured sea water. After 24 hours, the intestine is filled with a bright red solution of the dye, considerably more concentrated than the ingested sea water. The red color is due to the alkaline reaction of the intestinal fluids, and the increased concentration of dye is due, as will be shown later, to the absorption of water from the intestine. The stomach contents are invariably acid in reaction and the dye has never been observed to be concentrated there.

The fact that phenol red is not readily absorbed from the intestine leads to its extensive concentration as water and salts are absorbed. Thus, 24 hours after immersion in the tinctured sea water the dye is usually 3 to 5 times as concentrated in the intestine as in the ingested sea water. This fact is of great importance in showing that in addition to swallowing sea water, these fish absorb a large fraction of the ingested water by way of the intestine. By adding alkali to develop the full color of the dye and centrifuging to remove the solid matter and the precipitated Mg, it is possible to determine the extent of concentration (i.e., of water absorption) that has occurred. The following is an example of a typical experiment: The intestine of an eel was emptied as far as possible of fluid by inserting a catheter through the anus and pressing upon the abdomen. The anus was then closed

with a ligature to prevent the escape of any intestinal fluid and the urinary papilla was tied. Twenty hours after immersion in the tinctured sea water 2.3 cc. of fluid were removed from the stomach and intestine. To dilute the dye in this fluid to the same concentration as in the ingested sea water 10.0 cc. of water were required. Thus the fish must have swallowed 12.3 cc. of sea water and absorbed 10.0 cc. of water, during this period of time. A small amount of dye was present in the bile and urine; consequently the above figures for ingested and absorbed water are in error by being too small. If any sea water had been present in the gastro-intestinal tract at the beginning of the experiment this fluid would in addition undergo some absorption, and the residue should be deducted from the 2.3 cc. residue observed, so that errors from this source would likewise tend to reduce the figures for both the ingested and absorbed water. Consequently the above figures are by all considerations minimal for the conditions of the experiment.

Further evidence that sea water is normally swallowed by marine fish, and that water is absorbed from the intestinal tract will be given later in connection with the chemical composition of the gastro-intestinal fluids.

The validity of the above conclusions depends upon the exclusion of the possible though highly improbable alternative that the dye might have been absorbed by the skin or gills and excreted into the stomach and intestines. That this is not the case can be shown in *Anguilla* by ligating the pylorus to prevent the passage of sea water into the intestine. The body wall is opened opposite the lower end of the esophagus and the latter is tied in two places, a few millimeters apart, to insure complete obstruction. The incision in the body wall is then closed with ligatures and the fish placed in sea water heavily tinctured with phenol red. After 24 to 48 hours the fish is sacrificed and the stomach and intestinal contents tested for the dye by the addition of excess alkali. The invariable and complete absence of phenol red from the stomach and intestine in a large number of such experiments has convinced us that this dye gains access to the gastro-intestinal tract only in consequence of the passage of swallowed sea water through the stomach. If the phenol red remains for considerable periods of time and in a concentrated state in the intestine it may be absorbed, and it then appears in the bile as well as the urine, but this hepatic excretion occurs only after gastro-intestinal absorption, and never in the absence of dye from the gastro-intestinal tract. From a large number of experiments with starved but otherwise apparently normal fish we conclude that from 50 to 200 cc. per kilo per day of sea water are ingested by *Anguilla* and *Myoxocephalus* when these fish are immersed in sea water having a freezing point of $-1.8°C$.

*The extrarenal excretion of water.* The collection of urine in the sculpin and the eel is facilitated by the fact that the bladder is readily accessible

through the urinary papilla. The bladder may be completely emptied by inserting a fine glass catheter through the papilla and expressing the urine by massaging the abdomen over the bladder. When it is desired to collect all the urine excreted over a period of hours, the bladder may be emptied and then the urinary papilla tied to prevent discharge. Collections over too long a period of time should be avoided because of the danger of over-distention of the bladder.

To continue with the eel experiment cited above, 2.3 cc. of urine were excreted during the 24 hours that the fish was in the tinctured sea water. Since 10.0 cc. of water had been absorbed from the intestine, it follows that either *a*, 7.7 cc. of water had been retained by the fish, or *b*, this quantity of water had escaped from the body by some route other than the kidneys and intestinal tract. The eel weighed 143.5 grams at the beginning of the experiment and 142.2 grams at the end; it had, therefore, lost 1.3 grams body weight, presumably as water. Adding this loss in weight to the 7.7 cc. unaccounted for above, it follows that 9.0 cc. of water had been excreted extrarenally and 2.3 cc. excreted by the kidneys. Data to be given subsequently furnish further evidence of the extrarenal excretion of water. Meanwhile reference may be made to table 1 which gives the results of several experiments with *Anguilla* and *Myoxocephalus* performed as above. These experiments show that these fish swallow from 50 to 200 cc. of sea water per kilo per day, of which they absorb roundly 75 per cent of the water. Of the absorbed water, as much as 90 per cent may be excreted by routes other than the kidneys.

*Chemical composition of gastro-intestinal fluids.* We have not succeeded in obtaining sufficient fluid from the stomachs of normal *Anguilla* and *Myoxocephalus* for analysis, but ample fluid can be obtained from the intestinal tract for this purpose. In a few instances sufficient quantities of fluid were obtained from single specimens to permit complete analysis by using 0.2 cc. samples. Otherwise composite samples were analyzed. Data on the osmotic pressure and inorganic composition of intestinal fluids are given in table 2, and also analyses of fluids removed from fasted and fed specimens of the goosefish, *Lophius piscatorius*. The fasted *Lophius* were specimens caught off Sandy Hook by the New York Aquarium's boat *Sea Horse* and kept in the Aquarium in New York Harbor water for 3 to 5 days without food. The stomachs were empty and contained only a small quantity of clear, colorless, mucilaginous fluid. The contents of the intestines were also clear, though sometimes bile stained. The fed specimens had been freshly caught off Sandy Hook by local fishermen and all proved on inspection to have food masses in the stomach in a partially digested state. The upper intestinal contents were cloudy with food residues, while the lower intestinal contents were for the most part clear, except for granules of a mucilaginous nature. The intestinal contents are

designated as "anterior" or "posterior" according to the region from which they were removed. All fluids were centrifuged to remove particulate matter prior to analysis, and in the case of the fed *Lophius*, trichloroacetic acid filtrates were prepared from the gastric and intestinal fluids to remove partially digested protein. Where urine and intestinal fluids were removed from the same fish this fact is indicated by corresponding numbers. (It may be noted with respect to these data that the failure of the inorganic cations to balance the inorganic anions is attributable in part to the presence of proteinates, bile salts, ammonia and other organic substances, and that some carbonate as well as bicarbonate is probably present in the alkaline intestinal fluids.)

It will be convenient to refer first to the analyses of the fluids from *Lophius* because here we have simultaneous analyses of the gastric and intestinal fluids from the same specimens.

In the gastric contents of both fed and fasted *Lophius* Mg and $SO_4$ are more dilute than in sea water. As these fluids pass down the intestine, Mg and $SO_4$ invariably become concentrated 6 to 10 times (by the intestinal absorption of water) and when the lower intestine is reached the concentrations present are several times greater than in the Harbor water itself. As the Mg and $SO_4$ increase, the Na, K and Cl initially present in the gastric fluid fail to increase, or may even decrease in concentration, so that in the end Mg and $SO_4$ come to make up the bulk of the residual salt in the alkaline intestinal residue.

The osmotic pressure of the gastric fluid is invariably less than that of the sea water from which the fish are removed, but greater by a considerable amount than the osmotic pressure of the blood. The osmotic pressure of the gastric fluid, and the relative dilution of Mg and $SO_4$ in it, are explicable by the assumption that this fluid is a mixture of swallowed sea water of high osmotic pressure and gastric juice of an osmotic pressure approximately equal to that of the blood. In addition, it may be supposed that when sea water is taken into the stomach, the higher osmotic pressure of the former would lead to a withdrawal of water from the blood into the gastric contents, the sea water thus becoming appreciably diluted.

The progressive decrease in osmotic pressure in the intestine indicates that either water is moving into the intestine from the blood, or salts are being absorbed. It was not possible for us to determine by direct experiments on *Lophius* which of these alternative processes obtains; but the answer is clearly indicated by the rapidly mounting concentrations of Mg and $SO_4$ and the disappearance of Na, K, Ca and Cl. Between the stomach and the lower intestine, Mg and $SO_4$ are concentrated 6 to 1, or more. These substances occur in only small amounts in the bile, indicating that they are not extensively excreted by the liver, and we have shown in *Anguilla* (in which an entirely similar situation obtains) that they are not

secreted in significant amounts by the intestinal mucosa (*vide infra*). Consequently the above facts indicate the absorption of both water and salts, other than Mg and $SO_4$, from the intestine. The decrease in osmotic pressure is due in part to the fact that it is primarily the univalent salts which are absorbed leaving the osmotically less active bivalent Mg and $SO_4$ behind. In addition, it is possible that actually more salt than water, in osmotic equivalents, is taken up by the intestine.

TABLE 1

*Phenol red experiments showing intestinal absorption and extrarenal excretion of water by starved Anguilla and Myoxocephalus in sea water*

"Water absorbed" was determined by diluting dye in intestinal residue to its original concentration in sea water.

| CUBIC CENTIMETERS PER KILOGRAM PER DAY | | | | PER CENT | |
|---|---|---|---|---|---|
| Swallowed | Absorbed | Excreted in urine | Excreted extrarenally | Of sea water absorbed | Of absorbed water excreted extrarenally |
| *Anguilla* | | | | | |
| 50.0 | 37.0 | 16.4 | 20.6 | 74 | 56 |
| 135.5 | 113.0 | 9.9 | 103.1 | 83 | 91 |
| 52.0 | 39.0 | 7.8 | 31.2 | 75 | 80 |
| 40.0 | 30.0 | 10.0 | 20.0 | 75 | 67 |
| 54.4 | 46.0 | 4.6 | 41.4 | 85 | 90 |
| Average | | | | 78 | 77 |
| *Myoxocephalus* | | | | | |
| 225.0 | 169.0 | 41.6 | 127.4 | 75 | 76 |
| 89.5 | 44.1 | 16.8 | 27.3 | 63 | 62 |
| 109.0 | 58.3 | 25.1 | 33.2 | 55 | 57 |
| 59.3 | 44.5 | 37.6 | 6.9 | 75 | 16 |
| 96.8 | 43.0 | 28.0 | 15.0 | 45 | 35 |
| Average | | | | 63 | 49 |

The entire picture corresponds, in brief, to our idea of what would inevitably happen to swallowed sea water; namely, osmotic and chemical dilution in the stomach by admixture with gastric juice, followed by absorption of salts together with the osmotically equivalent water during passage through the intestine, the poorly absorbed Mg and $SO_4$ largely remaining behind in the intestinal residue.

The analyses of the intestinal fluids removed from *Anguilla* and *Myoxocephalus* give results entirely similar to those obtained in *Lophius*; the Mg

TABLE 2

*Composition of gastro-intestinal fluids in marine teleosts*

| | | $\Delta$ °C. | mM PER LITER | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | Na | K | Ca | Mg | Cl | $SO_4$ | $PO_4$ | $CO_2$ |
| **Fasted *Lophius*** | | | | | | | | | | |
| | Harbor water | 1.17 | 235 | 8.0 | 6.9 | 29 | 275 | 18 | 0.0 | 2.5 |
| 12 | Gastric | 1.03 | 198 | 3.9 | 6.2 | 30 | 235 | 17 | | 6.5 |
| | Ant. intest. | 0.93 | 140 | | 30.2 | 121 | 72 | 156 | | 59.0 |
| | Gastric | 1.00 | 255 | 9.7 | 6.0 | 16 | 246 | 18 | 0.9 | 15.0 |
| 13 | Ant. intest. | 0.97 | 137 | 43.2 | 11.2 | 75 | 109 | 93 | 5.5 | 50.0 |
| | Post. intest. | 0.84 | 140 | 11.4 | 6.6 | 99 | 81 | 114 | 1.0 | 55.0 |
| | Bile | 0.81 | 264 | 14.1 | 14.3 | 11 | 26 | | | 26.0 |
| | Gastric | 0.84 | 228 | 6.3 | 6.0 | 8 | 225 | 17 | | 3.0 |
| 14 | Ant. intest. | 0.76 | 103 | 4.8 | 19.5 | 85 | 88 | 126 | Tr. | 25.0 |
| | Bile | 0.58 | 178 | 15.0 | 6.6 | 7 | 56 | | | 20.0 |
| 15 | Post. intest. | 0.71 | 60 | 4.7 | 39.7 | 81 | 18 | 140 | | 53.0 |
| 16 | Post. intest. | 0.72 | 38 | 1.0 | 12.8 | 114 | 38 | 100 | | 75.5 |
| 17 | Post. intest. | 0.58 | 87 | 5.0 | 26.9 | 96 | 28 | 110 | 5.0 | 71.5 |
| **Fed *Lophius*** | | | | | | | | | | |
| | Sandy Hook water | 1.33 | | 4.6 | 8.0 | 33 | 350 | 26 | 0.0 | 2.0 |
| 18 | Gastric | 1.23 | 157 | 113.0 | 11.1 | 16 | 201 | 8 | 23.6 | 20.3 |
| | Ant. intest. | 1.05 | 159 | 83.0 | 14.8 | 92 | 89 | 94 | 8.6 | 26.2 |
| 19 | Gastric | 1.26 | 179 | 110.0 | 12.9 | 11 | 208 | 6 | | 20.5 |
| | Ant. intest. | 1.23 | 159 | 95.0 | 19.4 | 75 | 76 | 127 | 41.6 | 30.5 |
| | Gastric | 1.06 | 122 | 117.0 | 8.2 | 5 | 174 | 6 | 6.2 | 23.2 |
| 20 | Ant. intest. | 1.04 | 143 | 77.0 | 15.2 | 60 | 90 | 94 | 9.6 | 34.2 |
| | Post. intest. | 0.83 | 128 | 37.0 | 18.8 | 92 | 72 | 122 | 5.0 | 60.5 |
| | Gastric | 1.37 | 173 | 136.0 | 9.5 | 15 | 206 | 12 | 17.2 | 39.8 |
| 21 | Ant. intest. | 1.19 | 133 | 75.0 | 19.9 | 91 | 135 | 96 | 9.6 | 49.3 |
| | Post. intest. | 1.12 | 171 | 47.0 | 25.5 | 89 | 95 | 146 | 2.8 | 66.0 |
| 22 | Post. intest. | 0.70 | 116 | 16.0 | 12.8 | 74 | 123 | 70 | Tr. | 35.5 |
| | Bile | | 234 | 18.0 | 6.4 | 3 | 154 | | | 10.1 |
| **Fasted *Anguilla*** | | | | | | | | | | |
| | Sea water* | 1.81 | | 8.3 | 10.3 | 44 | 457 | 31 | 0.0 | 3.0 |
| 23 | Intestine | 1.26 | | 6.4 | 8.9 | 104 | 290 | 63 | 0.0 | 26.0 |
| 24 | Intestine | 1.40 | | 0.9 | 6.5 | 250 | 55 | 184 | 0.0 | 117.0 |
| 25 | Intestine | 1.20 | | 1.2 | 6.8 | 157 | 55 | 153 | 0.0 | 94.0 |
| 26 | Intestine | 0.75 | | 0.6 | 11.3 | 139 | 76 | 122 | | |
| 27 | Intestine | 0.69 | | 0.0 | 7.5 | 179 | 46 | 144 | | |
| **Fasted *Myoxocephalus*** | | | | | | | | | | |
| | Sea water | 1.85 | | 9.2 | 10.0 | 49 | 505 | 30 | 0.0 | 2.4 |
| 43 | Intestine | 0.75 | | 0.1 | 101.5 | 67 | 122 | 109 | 0.0 | 42.6 |
| 44 | Intestine | 0.82 | | 0.1 | 114.0 | 57 | 136 | 107 | 0.0 | 40.5 |
| 45 | Intestine | 0.72 | | Tr. | 127.5 | 56 | 119 | 112 | 0.0 | 31.1 |
| 46 | Intestine | 0.75 | | 0.2 | 72.0 | 70 | 133 | 87 | 0.0 | 28.0 |

* This sea water was used for no. 26 only, and for calculation of table 4. The other eels were kept in sea water differing but slightly from this sample.

and $SO_4$ contained in the swallowed sea water become concentrated by the intestinal absorption of water while the simultaneous absorption of Na, K and Cl results in a decrease in osmotic pressure so that the ultimate intestinal residue approaches the blood in tonicity.

The posterior portion of the intestine of *Anguilla* and *Myoxocephalus* is differentiated from the rest by being somewhat larger, and in the normal

TABLE 3

*Composition of marine teleost urine*

| | | °C. | mM PER LITER | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | K | Ca | Mg | Cl | $SO_4$ | $PO_4$ | $CO_2$ |
| 1 | *Lophius*, fasted | 0.69 | 1.9 | 11.8 | 63 | 172 | 40 | 7.1 | 1.5 |
| 2 | *Lophius*, fasted | 0.66 | 2.7 | 13.6 | 57 | 148 | 37 | 6.1 | 4.0 |
| 3 | *Lophius*, fasted | 0.72 | 2.7 | 15.3 | 74 | 173 | 41 | 6.1 | 2.0 |
| 4 | *Lophius*, fasted | 0.85 | 3.0 | 13.5 | 88 | 209 | 47 | 5.9 | 2.0 |
| 5 | *Lophius*, fasted | 0.77 | 0.6 | 15.5 | 73 | 195 | 38 | 6.3 | 1.1 |
| 18 | *Lophius*, fed | 0.61 | 1.9 | 16.8 | 64 | 171 | 29 | 4.2 | 1.5 |
| 19 | *Lophius*, fed | 0.76 | 2.1 | 9.3 | 79 | 181 | 40 | 2.8 | 1.0 |
| 20 | *Lophius*, fed | 0.64 | 7.6 | 14.8 | 75 | 161 | 36 | 14.7 | 1.2 |
| 21 | *Lophius*, fed | 0.80 | 4.3 | 15.2 | 68 | 207 | 36 | 2.9 | 1.3 |
| 22 | *Lophius*, fed | 0.71 | 2.3 | 13.3 | 100 | 206 | 47 | 9.8 | 1.0 |
| 26 | *Anguilla*, fasted | 0.66 | 5.7 | 7.5 | 75 | 76 | 52 | | |
| 27 | *Anguilla*, fasted | 0.75 | 2.7 | 17.3 | 91 | 214 | 34 | | 4.0 |
| 28 | *Anguilla*, fasted | 0.80 | Tr. | 20.7 | 150 | 134 | 105 | 1.9 | |
| 29 | *Anguilla*, fasted | 0.81 | Tr. | 18.9 | 167 | 203 | 105 | 10.4 | 0.5 |
| 30 | *Anguilla*, fasted | 0.68 | Tr. | 16.5 | 140 | 79 | 113 | None | |
| 31 | *Anguilla*, fasted | 0.69 | Tr. | 17.7 | 150 | 84 | 125 | None | 4.3 |
| 37 | *Myozoceph*, fed | 0.78 | 0.1 | 23.6 | 114 | 129 | 54 | 16.0 | |
| 38 | *Myozoceph*, fed | 0.76 | 0.1 | 39.0 | 142 | 165 | 61 | 78.0 | |
| 39 | *Myozoceph*, fed | 0.75 | Tr. | 26.6 | 109 | 129 | 37 | 35.7 | |
| 40 | *Myozoceph*, fed | 0.73 | 0.2 | 8.0 | 76 | 178 | 35 | 26.0 | |
| 41 | *Myozoceph*, fed | 0.77 | Tr. | 30.7 | 125 | 191 | 34 | 21.7 | 4.6 |
| 42 | *Myozoceph*, fed | 0.83 | 0.1 | 31.4 | 125 | 231 | 32 | 23.6 | 3.9 |

animal is sometimes distended with fluid residue. This "rectum" is separated from the anterior intestine by a band of highly tonic muscle which apparently acts as sphincter. It seemed possible that water absorption might occur here to a greater extent than in the rest of the intestine and that the osmotic pressure of the intestinal residue might actually be raised to a value greater than the blood in this limited region only, as is the case in the cloaca of birds and reptiles. For this reason several composite samples of fluid were removed by catheter through the anus with

special care not to expel fluid from the upper intestine. Analyses of two such samples are given in table 2, nos. 26 and 27, and analyses of the composite urine collected at the same time in table 3, no. 26. (These eels had been in sea water for 10 days and were presumably thoroughly acclimatized.) The fact that the osmotic pressure in these samples is low indicates that the fluid in this portion of the intestine is more nearly isotonic with the blood than that removed from higher regions, and that this rectal portion, like the rest of the intestine, is unable to absorb water against osmotic pressure.

In none of the higher animals, so far as is known, is the intestine capable of absorbing water against osmotic pressure, and consequently it is not surprising to find that in fish a similar situation obtains, in that the intestinal residue is isotonic, or approachs isotonicity with the blood. Nor is there anything unusual in the interpretation of the data of table 2 in terms of the more rapid absorption of Na, K and Cl from the intestine leaving the less readily absorbed Mg and $SO_4$ predominating in the residue; in this respect the fish again conforms with what is known about the higher animals (Goldschmidt, 1921). One might be content to let the matter rest with the above evidence, were it not that extraordinary conclusions necessarily follow from this interpretation and make it highly desirable to prove that no significant fraction of the Mg and $SO_4$ in the intestinal residue come there by secretion of the intestine itself.

This proof was adduced by ligation of the pylorus as described above. After the operation the intestine was thoroughly rinsed of its immediate $SO_4$ or Mg by forcing a NaCl solution ($\Delta = -0.8°$C.) into the anus with a rubber nipple pipette. This rinsing must be repeated a number of times at hourly intervals, for the mucosa is literally saturated with both $SO_4$ and Mg and these substances are removed with difficulty. The experiment was varied by using fresh water eels which had not been in sea water, and which had, therefore, less Mg and $SO_4$ in the intestinal mucosa to begin with. Phenol red was always added to the sea water to detect leakage of the pyloric ligature.

If the intestine of an unoperated eel is rinsed, the anus closed and the fish then placed in sea water, from 300 to 900 $\times 10^{-3}$ mM of $SO_4$ or Mg accumulate in the intestine in 24 hours due to the entrance and absorption of sea water. With the pylorus closed by ligature, no fluid whatever accumulates in the dry intestine, and the $SO_4$ or Mg that can be recovered by washing at the end of 24 hours ranges from zero to $60 \times 10^{-3}$ mM.

These experiments indicate that the Mg and $SO_4$ normally found in the intestine of salt water fish come there by the ingestion of sea water, and not by secretion by the intestine following their absorption into the body through the gills or skin. Another, and somewhat more satisfactory, type of experiment leading to the same conclusion is based on the fact that

*Anguilla* (as well as other fish) when in fresh water does not swallow water, the urine under these conditions being derived from water absorbed by the esophageal or branchial membranes. Thus, if the fish is placed in a simple N 0.05 $MgSO_4$ solution ($\Delta = -0.15°C.$), none of the solution is, as a rule, swallowed in 2 to 3 days and during this time the intestine remains free of both Mg and $SO_4$. If these salts were absorbed by the skin, etc., and secreted into the intestine, we see no reason why such absorption and secretion should not proceed from this solution as well as from sea water with the same concentration of $MgSO_4$.

*Composition of urine.* Turning now to the composition of the urine, given in table 3, it will be noted first that this is invariably isotonic or hypotonic to the blood, the freezing point of which in these fish varies from $-0.6$ to $-0.8°C.$

Thus, the osmotic pressure of the urine is considerably lower than that of sea water. This fact, as we remarked in the introduction, requires that the fish remove water from sea water against the osmotic pressure of the salts in the latter, a task which involves the expenditure of energy in the form of the physical-chemical work required to concentrate the corresponding quantity of sea water. To this urine there must be added the osmotically dilute intestinal residue, both of which must be obtained from the relatively concentrated sea water.

The mutually dilute nature of the urine and intestinal residue force us to the conclusion that osmotic work is being done by the fish elsewhere than in the kidneys and gastro-intestinal tract.

In regard to the inorganic composition of the urine, the most striking feature is the large amount of Mg and $SO_4$.[2] These substances may be, and usually are, present in the urine 3 to 6 times as concentrated as in the sea water. This fact is also shown by the analyses of the urine of marine teleosts made by Sulze (1922), Edwards and Condorelli (1928) and Marshall and Grafflin (1928).[3]

[2] The intestinal fluid and frequently the urine of marine fish contains solid matter in suspension which consists largely of Ca carbonates and $Mg(OH)_2$ in the former and Ca phosphates in the latter. This particulate matter was centrifuged out and only the supernatant fluid analyzed. (Guitel, 1906, has noted the presence of calcium concretions in the ureters of marine teleosts.)

[3] It is noteworthy that *Lophius* possesses a purely tubular kidney (Marshall and Grafflin, 1928), and the question is pertinent whether this fact makes any significant difference in the inorganic composition of the urine. It would appear that such is not the case, for Sulze's data show similar urine composition in the aglomerular *Lophius* and in *Conger* and *Scorpaena* which are predominantly glomerular (Marshall, 1929a). Edwards and Condorelli (1928) found Mg in amounts greater than in sea water in the urine of *Lophius* and of the aglomerular *Sygnathus* and *Hippocampus* and of the predominantly glomerular *Muraena*. Add to these observations our similar findings in the glomerular *Anguilla* and *Myoxocephalus* and the conclusion is justified that the osmotic pressure and inorganic composition of normal urine is not significantly dependent on the pressure or absence of glomeruli.

The presence of large amounts of Mg and Ca in the urine of fish has been especially noted by Sulze (1922), who concluded that marine fish do not swallow sea water, since he did not observe it in the stomach; and that the Mg and Ca excreted in the urine must, therefore, have gained access to the body through the skin or gills. The fact that Mg and Ca occur in much larger amounts in the urine than Na, K or Cl Sulze explained by assuming that the former enter the body much more readily than the latter.

*A priori*, there is much to argue against Sulze's view. There is not, in the first place, any evidence that Mg and $SO_4$ penetrate any tissue more readily than do Na, K and Cl. There is ample evidence to indicate that the reverse is true in higher organisms and that Mg and $SO_4$ are absorbed to only a very slight extent or not at all (Goldschmidt, 1921; Denis and Hobson, 1923; and Denis and Leche, 1925).

In refutation of Sulze's view it has been shown here that large amounts of sea water are swallowed by marine fish and that water, Na, K and Cl are absorbed into the body, leaving Mg and $SO_4$ in the intestinal residue. The failure, therefore, of Na, K and Cl to appear in the urine cannot be attributed to their failure to gain access to the body. Nor can the preponderance of Mg and $SO_4$ in the urine be attributed to preferential absorption from the intestine, for they are demonstrably absorbed less readily than Na, K and Cl. Setting aside, for the moment, the fate of the absorbed Na, K and Cl, and considering only the Mg and $SO_4$, it would seem that, in view of the enormous concentrations of these substances occurring in the intestinal residue as the result of the absorption of water and salts, some absorption of them must inevitably occur there. In this view it should be possible to exclude these salts from the urine by ligating the esophagus; but this operation in *Anguilla* effects only a moderate reduction in the urine concentration, or in the quantities excreted per day. The apparent reason for this is that with the ligature placed near the stomach (as it must necessarily be in *Anguilla* since the esophagus is not accessible higher up) the continued efforts of the fish to drink lead to marked distention and acute dilatation of the esophagus and in this dilated state, considerable quantities of sea water and its contained salts enter the body.

Since it is impossible to ligate the esophagus at the oral end without obstructing respiration, it is impossible to prevent absorption of Mg and $SO_4$ by operative procedures.

Evidence for the alimentary origin of the urinary Mg and $SO_4$ can be adduced, however, by using simple $MgSO_4$ solutions as described above. In 0.05 N $MgSO_4$ the urine flow is very great (from 40 to 100 cc./kgm./day) the water for the urine being absorbed elsewhere than from the stomach and intestine as demonstrated by the failure of the fish to drink tinctured sea water. When kept in such solutions, *Anguilla* rarely swallows during the first 2 to 3 days. The Mg and $SO_4$ in the urine are very dilute and the total

amounts excreted per day are considerably less than when the fish is kept in sea water. The average results from several experiments of this kind are given in table 4. The basal Mg and $SO_4$ excretion in fresh water was determined on fresh water eels which had never been (to our knowledge) in sea water. In these experiments, because of the greater urine flow, the urine was collected by fastening a retention catheter with a 50 cc. rubber balloon attached in the urinary papilla. In the $MgSO_4$ experiments, the urine samples were collected only on the second day. The figures for sea water are based upon the excretion of fish which had been in sea water but 48 hours, to keep the conditions comparable with the $MgSO_4$ experiments. The results of these experiments show that after 48 hours in sea water *Anguilla* excretes in the neighborhood of ten times as much Mg as when immersed for the same period of time in 0.05 N $MgSO_4$ solution, and if

TABLE 4

*A comparison of the quantities of Mg and $SO_4$ excreted in the urine by Anguilla when immersed in (1) fresh water, (2) N 0.05 Mg $SO_4$ solutions, $\Delta = -0.15°C.$, and (3) sea-water, $\Delta = -1.85°C.$*

The low excretion in (2) is presented as evidence against the absorption of Mg and $SO_4$ by the skin or gills.

| | | Mg | | $SO_4$ | |
|---|---|---|---|---|---|
| | | mM per liter | mM per kgm. per day $\times 10^3$ | mM per liter | mM per kgm. per day $\times 10^3$ |
| 1 | Fresh water | 0.7 | 70 | 1.3 | 130 |
| 2 | $MgSO_4$ solution | 2.19 | 82 | 6.0 | 223 |
| 3 | Sea water (average) | 150.0 | 750 | 100.0 | 500 |
| 4 | Sea water (diuresis) | 145.0 | 5,800 | 40.0 | 1,600 |

diuresis is produced by injecting sea water into the intestine through the anus, (*vide infra*) the difference may become still greater. The $SO_4$ excretion under the several conditions shows somewhat smaller differences, due apparently to the fact that less $SO_4$ was absorbed from the intestine during the experiment in sea water.

In another experiment of this same nature, after a control sample of urine was collected in distilled water, an eel was placed for three days in 0.01 N $MgSO_4$. When the total Mg and $SO_4$ excreted during the three day exposure to this solution was corrected by deducting the basal excretion, as determined during the control period, there remained $233 \times 10^{-3}$ mM. of Mg per kgm. per day excreted over the basal, but no $SO_4$. In a corresponding quantity of the $MgSO_4$ solution there were $1490 \times 10^{-3}$ mM. of Mg and $1639 \times 10^{-3}$ mM. of $SO_4$. In short, the absorption of water in this solution had occurred in some place and in such a way that 90

per cent of the Mg and 100 per cent of the $SO_4$ had been excluded from the body.

These experiments leave no doubt that when immersed in $MgSO_4$ solutions in which the concentration of this salt ranges up to that of sea water, neither Mg nor $SO_4$ enter the body to any great extent unless the fish swallows some of the solution. If these substances entered the body of the fish in sea water through the skin and gills, as Sulze supposes, it is difficult to explain why they should fail to do so from $MgSO_4$ solutions. In view of the fact that both Mg and $SO_4$ are to some extent absorbed from the intestine (*vide infra*) and in view of the enormous concentrations of these substances in the intestines of normal fish, as shown by table 2, we are forced to set Sulze's interpretation aside and to conclude that apart from a moiety of $SO_4$ of metabolic origin, the urinary Mg and $SO_4$ of marine fish enter the body by alimentary absorption.[4]

Having established that the intestinal Mg and $SO_4$ are derived from ingested sea water, and that the urinary Mg and $SO_4$ are derived from the alimentary absorption of these substances, we arrive at new evidence for the extrarenal excretion of water. For these substances are present in both intestinal residue and urine in greater concentrations than in sea water; this circumstance can only obtain if part of the water from the ingested sea water is disposed of elsewhere.

*Extrarenal excretion of salts.* With these facts established we can return to the fate of Na, K and Cl. From the preponderance of Mg and $SO_4$ in the intestinal residue it follows that relatively more Na, K and Cl are absorbed than Mg and $SO_4$; and from the preponderance of Mg and $SO_4$ in the urine it follows that less Na, K and Cl are excreted by this route than Mg and $SO_4$. The obvious inference is that the Na, K and Cl which are absorbed ahead of the Mg and $SO_4$ are being excreted by some route other than the kidney.

The absence of K from the urines of fed *Lophius* is particularly striking evidence of the extrarenal excretion of this substance. *Lophius* is a notorious meat eater, consuming in a single meal several pounds of fish. Though digestion is relatively slow in cold blooded animals (Riddle, 1909; Dobreff, 1927), it would be expected that the large amount of K ingested in a continued meat diet would lead to the ponderance of this substance in the urine, as is the case with mammals. K is demonstrably absorbed from the intestine of *Lophius* (table 2) yet no specimen of urine which we have

[4] Ca, on which Sulze places as much emphasis as Mg, we omit from consideration for the reason that this substance is largely precipitated as carbonates in the alkaline intestinal residue, and as phosphates in the urine. In our analyses, this precipitated Ca was centrifuged out, and consequently the amount of Ca left in solution, especially in urine, depends upon the acidity, phosphate content, interval between collection and centrifuging, etc., and has no significance.

examined has contained more than traces of this substance, even when the process of digestion is well advanced (table 3 "fed *Lophius*"). Since this appears to be the normal state of affairs, it does not seem likely that the absence of K from the urine is to be explained in terms of retention.

A simple calculation based on the total Mg and $SO_4$ jointly excreted in the urine and intestinal residue shows that large amounts of Na, K and Cl which are contained in the ingested sea water, and which are shown to have been absorbed from the intestine, remain unaccounted for in the urine. These substances have disappeared: either they are being retained by the animal in large amounts or they are escaping from the body by some route other than the kidneys. Since the analyses of the intestinal fluids and urines from fish obtained under perfectly natural conditions (*Lophius* and *Myoxocephalus*) agree in respect to the above facts, we conclude that retention is an inadequate explanation and turn to the other alternative, namely, that the univalent ions are being excreted by an extrarenal route. This fact becomes more significant if we recapitulate two previous conclusions drawn from the above data:

It has been shown by phenol red experiments with *Anguilla* and *Myoxocephalus* that water is excreted extrarenally. Further evidence of this fact is obtained in these fish and in *Lophius* by the increased concentrations of Mg and $SO_4$ in the urine and intestinal residue as compared with the ingested sea water.

It is shown that osmotic work is being performed by some tissue or tissues other than the kidneys and intestine in all three species by the facts that the urine and intestinal residue are more dilute than the sea water from which they are derived.

Now comes evidence, in the absorption of Na, K and Cl from the intestine in quantities relatively greater than Mg and $SO_4$, and the excretion of these substances in the urine in quantities relatively less than Mg and $SO_4$, that indicates that NaCl and KCl are being excreted extrarenally.

It is conceivable that these three processes might be going on independently in separate places or even in the same place, but it is difficult to believe that such is the case. There are only two ways in which osmotic work can be done by the fish: 1, to extract water directly from sea water (say at the gills) to the exclusion of, and against the osmotic pressure of the salts in the latter; or 2, to secrete a hypertonic solution from the body into the relatively dilute sea water. If, for the moment, we admit the first of these alternatives, then we are faced with the extraordinarily complex phenomena of the organism *a*, extracting water from sea water for the formation of dilute urine and intestinal residue, *b*, excreting water into sea water (as shown the phenol red experiments and the excretion of Mg and $SO_4$), and *c*, excreting salts into sea water (as shown by absorption of Na, K and Cl from the intestine and the absence of these substances from the urine),

all simultaneously at some extrarenal and extra-intestinal point. If, now, we consider the second possibility, namely, that the fish is doing its extrarenal osmotic work by the secretion of a solution hypertonic to sea water, it is obvious that both salts and water must be available for this secretion; and the more water and salts thus secreted, the less will the absolute hypertonicity of the solution with respect to the external sea water have to be. Thus the *extrarenal osmotic work*, the *extrarenal excretion of water* and the *extrarenal excretion of salts* become simultaneously identified with a single process in this latter view. It seems to us that there can be no choice but to attribute the three processes to the secretion of a hypertonic solution.

*Extrarenal osmotic work.* If the absolute amounts of salt and water excreted extrarenally could be determined, one could calculate the osmotic pressure of this hypothetical solution. The absolute amounts of salts and water excreted extrarenally comprise the difference between the amounts ingested on the one hand, and the amounts excreted in the urine and intestinal fluid on the other. The average volumes of urine and intestinal fluids in the experiments given in table 1 are 1.5 and 1.9 cc. respectively; the latter figure is no doubt slightly large because of sea-water originally present in the intestinal tract at the beginning of the experiment. Making allowance for this error it would appear that about equal volumes of urine and intestinal residue are passed in any period of time.

The actual quantities of Cl and water excreted extrarenally can be calculated if we assume that no Mg is excreted extrarenally. The $SO_4$ is less reliable for this purpose, since a fraction of the urinary $SO_4$ is of metabolic origin. Assuming that all the Mg comes from ingested sea water it follows that for each liter of urine and each liter of intestinal residue excreted, an amount of sea water equal to $\frac{\text{mM. Mg urine} + \text{mM. Mg intestine}}{\text{mM. Mg sea water}}$ in liters must have been swallowed. Of this swallowed sea water, only 2 liters of water (1 liter as urine and 1 liter as intestinal residue) are accounted for and the remaining water must have escaped from the body elsewhere.

When the unaccounted-for Cl is dissolved in the unaccounted-for water, the resulting solution is richer in Cl than the ingested sea water, as shown by the figures in table 5. Since NaCl or sea water solutions of equal Cl content have the same osmotic pressure, it follows that this hypothetical excretion is by so much hypertonic to sea water. As would be expected, the hypertonicity is relatively greater in the case of *Anguilla* and *Myoxocephalus* in sea water than in the case of *Lophius* in Harbor water, for the osmotic pressure of the urine remaining the same, a relatively greater fraction of salt must be excreted into the sea water than into the more dilute Harbor water for each liter of urine formed.

*Demonstration of the extrarenal excretion of salts.* Since a relatively large

quantity of well aerated sea water is necessary to keep a fish alive it seems improbable that the extrarenal excretion of a hypertonic solution in sea water can be directly demonstrated. The extrarenal excretion of individual salts by Anguilla can be demonstrated by direct experiment, however, when this fish is kept in fresh water. The demonstration of this process in sea water is made difficult by the large amount of salts initially present, but in view of all the indirect evidence, as set forth above, pointing to the extrarenal excretion of salts in sea water, we consider its demonstration in fresh water to be of equivalent value. It may be remarked here that it is just those salts—Na, K and Cl—that fail to appear in the urine in sea water that are excreted extrarenally in fresh water, and, conversely, those salts

TABLE 5

*Calculation of the fraction of water and chloride and the osmotic pressure in the extrarenal excretion, based on the total Mg excreted in one liter of urine and one liter of intestinal residue*

| | QUANTITY INGESTED | IN URINE | IN INTESTINE | NOT ACCOUNTED FOR | PER CENT NOT ACCOUNTED FOR | mM Cl PER LITER $H_2O$ EXCRETED | mM Cl PER LITER $H_2O$ INGESTED | O.P. RELATIVE TO SEA WATER |
|---|---|---|---|---|---|---|---|---|
| *Lophius*, average: | | | | | | | | |
| Water, liters | 5.75 | 1.0 | 1.0 | 3.75 | 65 | | | |
| Cl, mM | 1,580 | 178 | 41 | 1,461 | 86 | 390 | 275 | 1.42 |
| *Anguilla*, no. 26 only: | | | | | | | | |
| Water, liters | 4.85 | 1.0 | 1.0 | 2.85 | 59 | | | |
| Cl, mM | 2,220 | 76 | 76 | 2,068 | 93 | 726 | 457 | 1.59 |
| *Myoxocephalus*, average: | | | | | | | | |
| Water, liters | 3.62 | 1.0 | 1.0 | 1.62 | 45 | | | |
| Cl, mM | 1,828 | 171 | 125 | 1,732 | 95 | 1,070 | 505 | 2.12 |

which from other considerations we believe to be excreted by the kidneys—Ca, Mg, and $SO_4$ and $PO_4$—that are excreted in fresh water entirely by the renal route.

In these experiments with fresh water, a normal solution of various salts was injected into the intestine through the anus with a blunt hypodermic needle and the anus closed by a ligature. The salt solutions were made up with about 0.1 per cent phenol red so that any leakage of the salt solution past the anal ligature could be detected readily. A retention catheter with rubber bag attached was fastened in the urinary papilla and the fish washed thoroughly in tap water for 1 to 2 hours to remove any of the salt solution from the skin. The fish was then placed in 2 liters of distilled water, aer-

ated by compressed air, for 10 to 56 hours. At the conclusion of the experiment the water was evaporated and made up to 100 cc. This concentrated solution was then tested for phenol red and all experiments in which this dye was present in the water were discarded, since the presence of the dye showed that some of the salt solution had leaked past the anal ligature. In the absence of dye from the concentrated water, the latter, along with the urine removed from the catheter bag, was analyzed by the usual methods, using from 1 to 3 cc. samples. The quantities of salts present in the water and urine, as determined by multiplying the volume of these solutions by the concentration of each salt in them, are given in table 6 as millimols $\times 10^{-3}$.

It will be noted that almost all the NaCl and KCl are excreted into the water around the fish, while Ca, Mg, $SO_4$ and $PO_4$ are excreted by way of the urine. The renally excreted $SO_4$ takes with it large amounts of Na into the urine, and Mg and Ca take with them large amounts of Cl. Several samples of slime, which is freely given off when the fish is held in a net, were analyzed and showed consistently that this slime is not the route of excretion of the above salts. It contains practically no Na, K, Cl or $PO_4$, a small quantity of Ca and Mg and, when fresh, a small quantity of $SO_4$. During bacterial fermentation and subsequent evaporation more $SO_4$ is split off, accounting for the consistent presence of small amounts of this substance in the water around the fish. (This bacterial production of $SO_4$ can be reduced to some extent by adding small quantities of boric acid to the water.) Nevertheless the quantity of $SO_4$ excreted in the urine after giving $Na_2SO_4$ is so large relative to the quantity in the water, that we conclude that $SO_4$, like Ca, Mg and $PO_4$, is excreted exclusively by the kidneys.

This direct demonstration that NaCl and KCl are excreted extrarenally when the fish is in fresh water is striking corroboration of the indirect evidence that these salts suffer a similar fate when the fish is in sea water. The fact that this extrarenal excretion occurs in fresh water as well as in sea water is an important one, for it indicates that this process is fundamentally common to fresh water as well as marine fishes, and probably constitutes the mechanism by which the composition of the blood is regulated in the former as well as in the latter.

*The site of extrarenal excretion.* The experiments of Bert (1871), Sumner (1905) and Scott (1913) show that the gills are the principal route by which osmotic changes are effected in fish placed in salt or fresh water. In apparent contradiction to this conclusion is the work of Portier and Duval (1922). These investigators found that scraping the slime from the skin of *Anguilla* resulted in rapid changes in osmotic pressure of the blood when this fish was placed in solutions of varying salinity. They draw the conclusion that the skin plays an important part in the water exchange, but their results, we believe, are open to another interpretation. *Anguilla* is a smooth skinned

fish lacking fully developed dermal scales, and by scraping the skin one impairs, perhaps, the normal integumentary insulation and permits the movement of water along the natural osmotic gradient to occur across the skin. It may well be supposed that under these conditions the physiological machinery normally operating to maintain the osmotic pressure of the blood would be over-taxed. The fact that no dermal lymphatic system exists in fishes, as in the Amphibia, supports the belief that the skin of the former is largely a passive osmotic insulator. The facts that the majority of fresh and salt water fishes are armoured with impervious scales, and that this armoured condition was characteristic of the primitive vertebrates, make it fairly certain that the skin was primarily entirely passive in the regulation of water equilibrium, and that its participation is still passive in those fishes in which, like *Anguilla*, the scales have been reduced to subcuticular vestiges. The absence of salts in significant quantities from the slime of this fish, as reported above, shows that the specialized dermal glands are not involved in this problem, except in so far as they contribute to the maintenance of the integumentary insulation.

The physiological problem of maintaining the osmotic pressure and salt composition of the blood against the stress of fresh or salt water is one that has been faced by the vertebrates since the time of their evolution. It is clearly a major problem involving a significant amount of physiological labor. It is to be expected that the tissues responsible for this task should be in intimate relationship with the blood stream, and it is not improbable that from the earliest history of aquatic animals, this task should go hand in hand with the task of respiration. In this view, the enormous blood flow to the gills with their extensive dispersion of blood internally and free exposure to water externally, stands in marked contrast to any other integumentary organ. These facts, supported by the experiments of Bert, Sumner and Scott, lead us to believe that the gills constitute the tissues through which osmotic regulation of the blood, by means of hypertonic salt secretion, is effected.

Lastly, it may be remarked that from a chemical point of view it would appear to be just as feasible for the animal to extract the water necessary for the formation of urine directly from the sea water at the branchiae, without going through the circuitous process of absorbing both water and salts from the alimentary tract and then excreting the bulk of both by an extrarenal route. So far as osmotic pressure is concerned the same amount of work would apparently be involved in both methods, and in the former, in addition to reducing the total salt handled, the organism could passively exclude the Ca, Mg and $SO_4$ which, in the latter method, gain access to the blood in consequence of the enormous intestinal concentration required and which constitute an additional burden upon the kidneys. It cannot be argued that the direct extraction of water from a salt solution is physiologi-

cally impossible, for this process apparently occurs in the cloaca of some reptiles and the birds, and in the tubules of the mammalian kidney. The answer to why the latter method prevails does not rest, apparently, upon any inherent advantage in the method, but rather upon the evolutionary history of the regulation of body fluid composition in the vertebrates. The fact that extrarenal excretion of salts occurs in fresh water as well as salt water indicates, as we have pointed out above, that the fundamental process of physiochemical regulation is qualitatively the same in fresh water and marine fish. If we think of the marine teleosts as having been derived from fresh water ancestors, then it seems possible that the direction of this cycle is determined by this fact; for the primitive fresh water forms were organized to hold salt in their blood in spite of the fresh water that bathed the gills, and the branchiae were thus oriented in the direction of being hypertonic to the external medium. The marine fish, in excreting a hypertonic solution through the gills, is continuing qualitatively in the mode of its fresh water ancestors, the only difference being an absolute elevation of the branchial osmotic level with respect to the blood.

*Effects of marine habitat upon the kidney.* It has been stated above that *Anguilla* when in fresh water does not swallow significant quantities of this water. Numerous observations on *Cyprinus* and *Carrassius* kept in fresh water tinctured with phenol red show that in most instances no water is swallowed within 24 to 48 hours. Ultimately the dye does appear in the gastro-intestinal tract, and becomes highly concentrated in the intestine by the absorption of water. But the fact that water is not swallowed in detectable quantities within the first 24 hours stands in marked contrast to the situation in marine fish which invariably "drink" within 2 to 3 hours after being placed in the tinctured sea water. In a series of experiments in which *Anguilla* was placed in diluted sea water for 24 hours to permit acclimatization, prior to the addition of phenol red, we found that water was ingested within the next 24 hours in solutions having the same freezing point as the blood, and in many instances in solutions somewhat more dilute. From these experiments we conclude that the cycle of water movement typical of the marine fish first becomes necessary at osmotic pressures (external) slightly below that of the blood.

In spite of the failure of fish in fresh water to ingest water, the urine flow is much larger than in marine fish. Thus we find urine flows (calculated as cc. per kgm. per day) ranging from 60 to 150 cc. in *Anguilla*, *Cyprinus* and *Carrassius*. Since this water is not absorbed from the gastro-intestinal tract it must enter the body of the fish either through the oral or esophageal membranes or through the gills, probably through the first.

In contrast to the large urine flows in fresh water fish, the volume of urine excreted per day is greatly reduced in marine fish. The following values (calculated as cc./kgm./day from the authors' data if not so expressed)

have been given by various investigators: *Lophius*, 20 to 30 cc., *Scorpaena*, 10 to 12 cc., *Conger*, 3 to 5 cc., (Burian, 1908); *Lophius*, 18 cc. *Muraena*, 5 cc. (Edwards and Condorelli, 1928);[5] *Lophius*, 13 to 54 cc., *Cryptacanthodes*, 1.5 to 11 cc., *Myoxocephalus*, 3.2 to 57 cc., *Opsanus*, 0.6 to 9.4 cc., (Marshall, 1929b). We have obtained under conditions as nearly normal as possible, from 11 to 40 cc. in *Myoxocephalus* and 0.4 to 5 cc. in *Anguilla*. We regard these figures as probably all abnormally high, for the reason that we have found that introducing sea water into the intestine of *Anguilla* increases the urine flow, as compared with a control period, on an average 10

TABLE 6

*The extrarenal excretion of salts by Anguilla after alimentary absorption*

(B = quantity present no greater than blank)

| SALT | HOURS | | VOLUME, CC. | mM × 10³ EXCRETED | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | Na | K | Cl | Ca | Mg | $SO_4$ | $PO_4$ |
| NaCl | 56 | Urine | 41 | 41 | 287 | 398 | 24 | 24 | 119 | 97 |
| | | Water | 2,000 | 1,530 | 420 | 1,950 | B | 84 | (100) | Tr. |
| KCl | 48 | Urine | 26 | 161 | 370 | 690 | 21 | 10 | 43 | 52 |
| | | Water | 2,000 | 2,120 | 1,900 | 2,360 | B | 27 | (150) | Tr. |
| $CaCl_2$ | 29 | Urine | 11 | | 60 | 828 | 275 | 36 | 75 | 129 |
| | | Water | 2,000 | | 183 | 600 | B | 24 | (77) | Tr. |
| $MgCl_2$ | 30 | Urine | 30 | 510 | 51 | 2,600 | 89 | 1,500 | 273 | 250 |
| | | Water | 2,000 | 306 | 330 | 750 | B | 62 | (62) | Tr. |
| $Na_2SO_4$ | 28 | Urine | 16 | 3,300 | | | | | 1,557 | |
| | | Water | 2,000 | 300 | | | | | (120) | |
| Control | 16 | Urine | 11 | 26 | 52 | B | 2.7 | 2.5 | 24 | 40 |
| | | Water | 2,000 | B | 304 | B | B | 46 | B | Tr. |
| Slime | | | | B | 17 | B | 15 | 17 | 25 | Tr. |

times, and may increase it 30 times. Unquestionably any increase in the quantity of water swallowed by the fish would have the same effect. Since a fish is always excited when it is handled or removed from water in a net, there is strong probability that removal from water leads to increased water ingestion and hence to increased urine flow. Grollman (1929) has noted

[5] Edwards and Condorelli give much larger figures for *Sygnathus* and *Hippocampus*, but since these fish are very small and since these investigators placed a ligature entirely around the body and subsequently collected the urine by cutting the bladder over a test tube, the figures must be set aside as questionable.

that the urinary N of Lophius urine obtained from fish freshly caught off the Grand Banks is 2 to 3 times as great as in urines obtained from this fish when kept in captivity (Marshall and Grafflin, 1928; Edwards and Condorelli, 1928), indicating that in the latter case the fish are diuretic. It is possible that starved fish swallow an excessive quantity of sea water, and therefore have an excessive urine flow, as compared to fed fish. With these facts in mind, we believe that the *normal* urine flow of marine fish should be put at least as low as 5 per cent of that of fresh water fish, and possibly as low as one per cent.[6]

This difference is attributable to the facts that in salt water, in addition to the absence of any natural osmotic tendency for water to enter the body, a large fraction of all the water absorbed from the alimentary tract is excreted extrarenally. Furthermore, every liter of sea water handled by the marine fish is procured at the expense of considerable osmotic work in the extrarenal disposal of the contained salts, so we would expect the volume of ingested sea water to be kept as low as possible for reasons of physiological economy.

In the light of these facts it is significant that many fishes are known to possess partly or completely aglomerular kidneys (Marshall, 1929a) and that these aglomerular fish are all strictly marine. It seems probable that this aglomerular condition is a consequence of alterations in water economy incidental to the assumption of a marine habitat.

The portion of the kidney which has come, through a variety of evidence, to be viewed as primarily involved in the excretion of water is the glomerulus. Thus the modern theory of urine formation sponsored by Cushny is based upon the primary filtration of colloid-free plasma through the capillary tuft of the glomerulus, and the reabsorption of water and other constituents of this filtrate by the tubules. Though no incontrovertible evi-

[6] At first sight it would appear that all the water excreted as urine in marine fish might be accounted for as having a metabolic origin. Thus Magnus-Levy (quoted by Rowntree, 1922) gives the following figures for metabolic water derived from 100 grams of food: fat, 107 grams, starch, 55 grams, protein, 41 grams. Taking 100 cc. $O_2$/kgm./hr. as an average metabolic rate in active fish (Kestner and Plaut, 1924) and assuming that 1 gram of fat requires 2019 cc. of $O_2$ and 1 gram of protein requires 967 cc. of $O_2$ for combustion, one obtains from a pure fat diet 1.27 cc. of water, and from a pure protein diet 1.02 cc. of water per kilo per day. To the above must be added 0.8 cc. of water for each gram of body tissue degraded. These figures represent about the minimal rate of urine excretion of *Anguilla* in sea water. But in spite of the apparent adequacy of this explanation, there remain the facts that upwards of 50 cc. of urine per kilo may be excreted by *Anguilla*, *Myoxocephalus* and *Lophius* if intestinal absorption is great, and that the Mg (and $SO_4$) *normally* excreted in the urine of all fish in which this has been analyzed requires the extrarenal excretion of an amount of water equal to or several times as great as the urine itself. So that even under conditions of minimum urine flow the metabolic water in starved fish can hardly play a significant part in influencing the water economy as a whole.

dence is available to that effect, the volume of the urine is presumably determined by the volume of the glomerular filtrate minus such water as may be readsorbed by the tubules.

Abundant evidence indicates that the early evolution of the vertebrates occurred in fresh water, and that the marine fishes have migrated into the ocean in comparatively recent times. (This evidence is in part reviewed by Lull (1918) and other texts of biology and need not be detailed here.) In this view the glomerular kidney may be considered to be the older or fresh water type, and the degeneration of the glomeruli in marine fishes may be viewed as a secondary specialization.

That the absence or degeneration of glomeruli is not attributable to the extrarenal excretion of nitrogenous waste products is indicated by the fact that in fresh water fish the glomeruli are fully developed, although the greater portion of these substances are excreted by the gills (Smith, 1929). Nor can the aglomerular condition be attributed to the extrarenal excretion of salts. In fresh water fish, the renal salt excretion is primarily less than in the marine fish because of a smaller salt intake, and since there is an abundance of water available, these salts are excreted in very dilute solution. In the marine fish the salt excretion is greatly increased by virtue of the salts which are constantly being absorbed from the intestinal tract, and, moreover, a limited amount of water is available for their excretion. If the glomeruli were related primarily to the excretion of salts, then one would expect to find increased development of glomeruli, rather than reduction, in marine fish, for it is upon them that the greatest burden of salt excretion falls, both in respect to the absolute amounts of salts excreted and the concentrations of them in the urine.

The remaining factor is the excretion of water. From evidence cited above it appears that marine fish are faced with a permanent anuria relative to their fresh water ancestors, and it seems probable to us that this fact is responsible for the aglomerular condition.

If this interpretation is correct, aglomerular kidneys should never be found in fresh water fish, or in fish which migrate for any length of time into fresh water; and it may be supposed that it will be in those forms which have been longest in the sea, and which are therefore most highly specialized in directions characteristic of a marine habitat, that the glomerular degeneration will be most marked.

A study, similar to the above, of the salt and water equilibria in the marine cyclostomes and elasmobranchs will be reported in the future.

## SUMMARY

It is shown that marine teleosts swallow relatively large quantities of sea water which subsequently undergoes absorption. Most of the ingested water, Na, K and Cl are absorbed from the intestine leaving a residue rich in Mg and $SO_4$ and approximately isotonic with the blood.

The salts of the urine consist primarily of Mg and $SO_4$, with only traces of K and small quantities of Na and Cl, the solution being isotonic or hypertonic to the blood.

It is shown that the intestinal Mg and $SO_4$ come from ingested sea water, and that the urinary Mg and $SO_4$ come from alimentary absorption of these substances.

Quantitative analysis of the composition of the ingested sea water, the intestinal residue and the urine lead to the following conclusions:

1. The osmotically dilute nature of the intestinal residue and urine elaborated from sea water shows that the marine fish is doing osmotic work at some point other than in the kidneys and gastro-intestinal tract.

2. The fact that Mg and $SO_4$ occur in greater concentrations in both the intestinal residue and the urine than in the ingested sea water from which these fluids are derived shows that part of the water absorbed from the alimentary tract is excreted by some route other than the kidneys.

3. The fact that relatively more Na, K and Cl are absorbed from the alimentary tract than Mg and $SO_4$, while less Na, K and Cl are excreted in the urine than Mg and $SO_4$ shows that a large part of the absorbed NaCl and KCl are being excreted by some route other than the kidneys.

By considering these three processes as identified with a single process, it is inferred that the marine fish excretes by some extrarenal route a solution of NaCl and KCl which is *hypertonic* to the ingested sea water and thus leaves part of the absorbed water free for the formation of the osmotically dilute urine and intestinal residue.

The extrarenal excretion of water is demonstrated in *Anguilla* and *Myoxocephalus* by the addition of phenol red to sea water and the quantitative, simultaneous determination of the quantity of water ingested and the quantity of urine excreted in a given time.

The extrarenal excretion of salts is demonstrated in the catadromous eel, *Anguilla*, when kept in fresh water. The greater part of the NaCl and KCl absorbed from the alimentary tract is excreted by the extrarenal route, while essentially all the Ca, Mg, $SO_4$ and $PO_4$ are excreted by the kidneys. In the absence of other salts, $SO_4$ takes Na into the urine with it, and Mg and Ca take Cl.

Reasons are given for believing that the extrarenal excretion of salts and water and the extrarenal performance of osmotic work are attributable to the gills.

The water for the formation of urine in fresh water fish is not absorbed from the stomach or intestine, but probably from the oral membranes.

The water cycle in marine fish is of such a nature as to reduce the quantity of water excreted as urine to a small fraction of that excreted in fresh water fish. It is suggested that this relative anuria is the principal causative factor in an evident tendency for the glomeruli to disappear in the kidneys of strictly marine teleosts.

METHODS. The above conclusions are based on analyses of urine and gastro-intestinal fluids removed from fasted and fed fish under natural and experimental conditions supplemented by special experiments. All fluids were centrifuged to remove particulate matter, if present, prior to analysis.

Determinations of $CO_2$, Cl and freezing point were carried out directly upon the urine and intestinal fluids. $CO_2$ was determined by the manometric method of Van Slyke and Neill (1921) and Cl by the method of Van Slyke (1923). In the determination of Cl the nitric acid digest as well as the blanks and standards were routinely boiled for a minute or so over a microburner to expel all nitrous oxide fumes; they were then cooled under the tap and titrated at once. Midway of the titration 1 to 2 cc. of ether were added to cause the AgSCN precipitate to flocculate, the mixture was cooled if necessary and titration continued with vigorous shaking. With these precautions the end point is sharp and permanent.

A trichloracetic acid filtrate was prepared from the intestinal fluids of *Lophius* for the determination of $PO_4$ and $SO_4$; otherwise these substances were determined directly, $SO_4$ by Fiske's method (1921) after acidification to a pink color with methyl orange, or gravimetrically as $BaSO_4$ and $PO_4$ by Briggs' modification of the method of Bell and Doisy (1924).

Na, K, Ca and Mg were determined in redissolved aliquots after ashing with $H_2SO_4$, $HNO_3$ and superoxol. In the intestinal fluids from the fed *Lophius* the protein was first precipitated with trichloracetic acid, and the filtrate ashed. Otherwise the ashing was done directly. Na was determined by Kramer and Gittleman's method (1924), 0.5 cc. 10 per cent KOH being added prior to the addition of the pyro-antimonate reagent to precipitate the Mg. K was determined by precipitation as the cobaltinitrite (Kramer and Tisdall, 1921) the reagent being prepared as described by these authors. When prepared by this method we find the reagent keeps indefinitely at room temperature, whereas the commercial sodium cobaltinitrite powder has invariably decomposed and otherwise proved untrustworthy in our experience. At least 4 hours were allowed for complete precipitation and where the K concentration is small, the mixture is allowed to stand overnight. The potassium cobaltinitrate precipitate was measured by diazotization (Briggs, 1923) which we find much simpler and more reliable than permanganate titration. Thirty minutes were allowed for the development of color after diazotization. In the earlier experiments Ca was determined by Clark and Collip's method (1925) and Mg was determined in the supernatant fluid by precipitation as $MgNH_4PO_4$ (Denis, 1922) and determination of the $PO_4$ (Briggs, 1924). Later, a modified method communicated to us by Dr. Kenneth Blanchard, was introduced. This method appears to precipitate the Ca more effectively and to make a cleaner separation of the Ca and Mg:

Three cubic centimeters of a solution consisting of 50 cc. N HCl, 1.66 grams oxalic acid and 13.3 grams ammonium oxalate made up to one liter with water are added to the unknown in a 15 cc. pyrex centrifuge tube. The mixture is heated in a boiling water bath for 30 minutes, cooled and 0.2 cc. 20 per cent sodium acetate are added. After one hour the Ca oxalate is centrifuged out and the supernatant fluid decanted into 30 cc. pyrex centrifuge tubes for the determination of Mg. The Ca oxalate is washed twice with 2 cc. of 0.5 per cent ammonia solution, the first washing being added to the Mg. The Ca oxalate is then titrated with 0.02 or 0.01 N $KMnO_4$ solution after the addition of 2 cc. N $H_2SO_4$. The Mg is precipitated from the combined supernatant fluid and first washing from the Ca as $MgNH_4PO_4$. The solutions are heated in the water bath for 10 minutes and 1 cc. of 4 per cent $(NH_4)_2HPO_4$ and 2 cc. of concentrated ammonia are added. After standing overnight the precipitated phosphate is centrifuged out, the supernatant fluid decanted, the precipitate washed three times

with 50 per cent ammonia solution and dissolved in a known volume of 0.1 N HCl. Phosphate is determined in an aliquot of this solution by Briggs' (1924) method.

Both Mg methods were checked in many cases by weighing the $MgNH_4PO_4$ obtained from duplicate samples. Freezing points were determined with a Beckmann thermometer in an apparatus designed to work with 1 to 3 cc. of fluid. The accuracy of the method was checked against NaCl solutions of known composition. The figures given are uncorrected for under-cooling, which may be excessive in these fluids, and an allowance of $\pm 0.02°C.$ should be made for error from this source.

In the analyses of the fluids from *Lophius* 1 cc. or larger samples were used, except for $CO_2$ which was determined in a 0.2 cc. sample measured with a Van Slyke-Ostwald pipette. In most of the analyses of the fluids from *Anguilla* and *Myoxocephalus* it was necessary to use 0.2 cc. samples, measured with standardized wash-out pipettes.

For experimental work we have used the marine sculpin, *Myoxocephalus octodecimspinosus*, the catadromous eel, *Anguilla rostrata*, the fresh water carp, *Cyprinus carpio*, and gold fish, *Carassius auratas*. The sculpin experiments were done at Salisbury Cove with fish caught off the laboratory wharf by hook and line; the eel, carp and goldfish experiments were done at New York with fish purchased in the local markets and kept in running fresh water or aerated tanks of salt water in the laboratory. The eels were placed in fresh water for a day or more after bringing them into the laboratory and then transferred to salt water in which they were kept for 5 to 10 days, to acclimatize them before use. The eel is a fairly hardy fish and stands transfer from fresh to salt water well. It may be noted that this fish migrates to mid-ocean to spawn, and therefore a salt water habitat is not unnatural for it. It is admirably suited to experimental work, and there is no reason to believe that results obtained upon it are not generally applicable to other marine or fresh water fish. It may be remarked, however, that the mortality in experimental work of this kind is very great, and because of the inherent difficulties involved, a larger fraction of well planned experiments may be defeated by mechanical failures than is generally the case.

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# EXPERIMENTS ON THE ISOLATED WHOLE GALL BLADDER OF THE DOG

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The reactions of the gall bladder to various stimuli have frequently been investigated. Doyon (1) was the first to register graphically what he believed to be contractions of the gall bladder. Doyon and subsequently Courtade and Guyon (2), Freese (3), Bainbridge and Dale (4), Macht (5), Taylor and Wilson (6), Copher, Kodama and Graham (7), Brugsch and Horsters (8), Burget (9), and Ivy and Oldberg (10), experimented on anesthetized animals, mostly dogs, with the gall bladder in situ. Okada (11), in order to avoid anesthesia, worked on cholecystostomized dogs. Methods for studying pressure changes in the gall bladder of unanesthetized dogs were developed by Potter and Mann (12), McMaster and Elman (13), and by Wakerlin (14). It is not surprising that the results thus far obtained are in variance when the methods employed are critically viewed. It is also well to recall that Bainbridge and Dale (4) by inserting between the lobes of the liver a phantom gall bladder—a rubber balloon filled with fluid and connected with a writing tambour—discovered that stimulation of the nerve supply to the gall bladder caused much the same effect on the phantom gall bladder as on the real one. Copher, Kodama and Graham (7) registered rhythmic contractions in an unanesthetized dog by placing a small rubber bag into its gall bladder—but a rubber bag placed in the upper abdomen away from the gall bladder registered similar movements.

Another group of investigators worked on the isolated gall bladder and obtained more uniform results. Doyon (15) is credited as being the first to have observed spontaneous contractions of the isolated gall bladder. He reported that if the liver and the gall bladder be excised and placed in a warm, moist chamber, the gall bladder continues to contract for several hours. He did not, however, register these contractions graphically. Lieb and McWhorter (16) were the first to study the reactions of strips, longitudinal and circular, of the dog's gall bladder suspended in Ringer's solution at body temperature. They registered spontaneous rhythmic contractions and found that drugs which are recognized as stimulants of the parasympathetic nervous system, viz., pilocarpine and physostigmine,

produced contraction of the strips. The effect of atropine, which is supposed to paralyze the nerve endings of the parasympathetic nervous system, seemed to depend upon the degree of tonus at the moment of application: when it was low, atropine produced little or no effect; when increased it led to relaxation. Epinephrine, which is supposed to act on the myoneural junctions of the sympathetic nervous system, caused a loss of tonus with some decrease in the rate of contractions. Nicotine, which is supposed to act on the ganglia, produced a slight reduction in the rate of contractions and a considerable decrease in their strength in concentrations higher than 1:2000. Of the drugs which act particularly on smooth muscle, Lieb and McWhorter studied the effects of nitrites, bile salts, strophanthin, barium chloride and morphine. They observed that, like other smooth muscle, that of the gall bladder was depressed by nitrites (nitroglycerin and the sodium and amyl nitrites) which affected the tonus while the strength and rhythm of the contractions were not essentially altered. Sodium glycocholate and ox bile, caused a marked relaxation of the muscle with a weakening and slowing of the spontaneous contractions. Strophanthin and barium chloride produced an increase in tonus and also in the rate and strength of the individual contractions. Morphine, in concentrations between 1 and 5:100,000, produced either no effect at all or a very slight loss in tonus. With high concentrations 50:100,000 and above, the rate and strength of the contractions were usually decreased, the strength being more affected than the rate.

Macht (5) studied the effects of some opium alkaloids on isolated strips of the gall bladder of cats, dogs and pigs suspended in warm oxygenated Locke's solution. Macht confirmed the results of Lieb and McWhorter in regard to the effects of atropine and morphine. Methyl-morphine or codeine, and dimethyl-morphine or thebaine, produced increase in tonus of the isolated strips. Papaverine, narcotine and narceine were all found to decrease the tonus and to inhibit slightly the contractions, papaverine being the most effective of the group. A study of the effects of combinations of representatives of the morphine and papaverine groups showed in every case the predominance of the papaverine group. One part of papaverine, for example, could overcome the effect of five times its dosage of morphine, and pantopon or pantopium always tended to decrease the tonus, thus indicating the predominance of the papaverine effect.

Ischiyama (17) studied the reactions of the isolated whole gall bladder of the dog, as well as of strips suspended in oxygenated Ringer's, Ringer-Locke's or Tyrode's solutions at a temperature of 37°–38°C. In the experiments with the whole gall bladder, the viscus was freed of its content, rinsed and then filled with Ringer's solution and a cannula, connected with a writing tambour, was tied into the cystic duct. The strips—longitudinal or circular—used, were 3 to 4 cm. long and 1.5 cm. broad and

sometimes freed of their mucosa and serosa. Ischiyama registered spontaneous contractions of the whole gall bladder of the dog occurring at a rate of about 1 to 3 per minute. These spontaneous movements were about the same whether the viscus was removed under morphine, chloroform-ether anesthesia or without any anesthesia. The tonus decreased and the movements became sluggish at temperatures above 40° and below 36°C., and also with inadequate supply of oxygen. Of the chemicals acting on the parasympathetic nervous system pilocarpine, physostigmine, and muscarine increased the tonus and decreased the rhythmic movements; small doses of atropine relaxed the contraction caused by pilocarpine or muscarine, moderate doses increased the rhythmic movements, large doses decreased the tonus and stopped all movements. Of the chemicals acting on the sympathetic nervous system: adrenalin caused a decrease of tonus and cessation of the spontaneous rhythmic movements but in some instances they were both increased. Nicotine in doses of 1:15,000 to 20,000, increased slightly the tonus and the amplitude of the movements. Of the chemicals acting directly on smooth muscle barium chloride increased the rhythmic movements as well as the tonus. Secretin increased the tonus, leaving the spontaneous movements unaltered. Gastric juice increased the tonus; pancreatic juice, pancreatin, salt (sodium chloride?) showed no effect. Choline increased the height of the rhythmic movements. A choline-like substance extracted from the gall bladder wall produced an increase of the tonus as well as of the rhythmic movements, both of which were inhibited by atropine. The presence of choline or the choline-like extract of the gall bladder wall was found to modify the action of adrenalin upon the isolated gall bladder: the tonus and rhythm augmented by these substances were raised by adrenalin, while adrenalin alone diminished both the tonus and the rhythmic movements.

Chiray and Pavel (18) experimented on the isolated whole gall bladder of the dog, opened at both ends and suspended in a bath of Ringer's solution to which defibrinated dog's blood was added in a ratio of two parts of Ringer's solution to one part of dog's blood. The spontaneous movements registered by these authors were not dependent on the temperature: at 14°C. they were the same as at 45°C. Temperatures above 45°C. caused a sudden increase in tonus. Pilocarpine and eserine increased the amplitude and duration of the tonic contractions, which were not affected by atropine. Occasionally, however, isolated sudden contractions of high amplitude occurred which were relaxed by atropine. Adrenalin as a rule had no effect but at times it lowered the amplitude of the spontaneous movements.

Brugsch and Horsters (8) studied the effects of various drugs—hypophysin [Höchst], neohormonal [Zülzer], acetylcholine, atropine, adrenalin, gynergen, physostigmine—on the guinea pig's isolated whole gall bladder.

suspended lengthwise in an oxygenated Tyrode's solution at 37°C. Acetylcholine caused contraction and hypophysin relaxation of a circular strip, 0.5 cm. broad, of a guinea pig's gall bladder. A longitudinal strip, 2 mm. broad and 5 cm. long, of a dog's gall bladder suspended likewise in an oxygenated Tyrode's solution at 37°C. contracted when acetylcholine and relaxed when atropine and hypophysin were added. No reaction was obtained by these investigators on circular strips of a dog's gall bladder with either acetylcholine, histamine, pilocarpine, or hypophysin and atropine.

Mellanby (19) writing on the mechanism of pancreatic secretion states that "If strips of gall bladder are suspended in warm oxygenated Ringer a well-marked contraction is produced by adding a trace of pure secretin to the fluid."

Erbsen and Damm (20) studied the reactions of the guinea pig's isolated whole gall bladder suspended lengthwise or crosswise in an oxygenated Tyrode's solution at 38°C. Gall bladders of dogs and rabbits were also used but only as controls. They registered contractions with pilocarpine and relaxation with atropine and papaverine. Adrenalin relaxed the tonus, gynergen had no effect. Histamine caused contraction, guanidine was ineffective. Of the kations: barium (most potent) and potassium caused contraction, calcium and magnesium relaxation. The contraction produced by histamine was increased by barium. Of the hormones examined: the relaxed viscus was not influenced by the hypophysis hormone; the contracted organ relaxed or remained uneffected by hypophysin and pituitrin; thyroxin and insulin had no influence on the tonus.

Erbsen (21) reported studies on the effects of pituitrin and some narcotics on the guinea pig's isolated whole gall bladder suspended lengthwise in an oxygenated Tyrode's, Locke's or Ringer's solution of 37°C. Pituitrin and hypophysin caused the distended gall bladder to contract but had no effect on the empty viscus. Optimum effects were obtained with pituitrin in Ringer's solution. The distended gall bladder which was caused to contract by pilocarpine or histamine was not further affected by the hypophysis hormone. Application of narcotics prevented the tonus increasing effect of the hypophysis hormone. Of the narcotics: ether, cocaine, novocaine, and in a less degree urethane lowered the tonus; chloroform caused contraction of the relaxed and relaxation of the contracted gall bladder; morphine produced no change in tonus, but prevented the action of other stimuli.

Kalk (22), too, experimented on the guinea pig's isolated whole gall bladder suspended lengthwise in an oxygenated Tyrode's solution at body temperature. The distended gall bladder usually registered one to two slight rhythmic contractions per minute. Pituglandol, hypophysin [Höchst], and pituitrin [Parke-Davis] increased the tonus of the distended

viscus. The increase of tonus produced by histamine was not further influenced by hypophysin. The same gall bladder after its content had been removed registered no spontaneous movements and the rise in tonus produced by pituglandol and pituitrin was hardly noticeable. Pilocarpine produced a slow increase in tonus of the distended gall bladder, which was relaxed by adrenaline. Further doses of adrenaline gradually increased the tonus which was relaxed by atropine. Ergotamine (gynergen) caused a slow increase in tonus, which was relaxed by adrenaline. Pilocarpine increased the tonus of the gall bladder freed of its content. This increase in tonus, however, was less than that produced on the distended organ.

According to the available information Ischiyama (17) was the first, and the only one, to experiment on the isolated whole gall bladder of the dog. After removal, he freed the viscus from its content, rinsed and then filled it with Ringer's solution, and connected a cannula tied into the cystic duct with a writing tambour. This method of Ischiyama is far superior to any method previously employed for the study of the reactions of the isolated whole gall bladder, because the movements thus registered represent changes in the inner volume of the viscus. Ischiyama, however, had apparently not realized the opportunities offered by this method in obtaining valuable information regarding the function of the muscular coat of the biliary vesicle. It occurred to us that by constructing a cannula permitting accurate estimation, at any time, of the fluid content of the viscus and the measuring of the movements in terms of hydrostatic pressure, data on the motor mechanism of the gall bladder could easily be gathered which in view of the controversy regarding the contractile powers of the biliary vesicle would be most illuminating. Our expectations were fully realized by adopting and carrying out the following procedures.

METHOD AND APPARATUS. The animal was anesthetized with ether, the gall bladder removed, emptied, and its content measured.[1] A cannula, such as shown in figure 1, was tied into the neck of the gall bladder and the preparation mounted in an oxygenated bath of Locke's solution of approximately 250 cc. capacity. This bath was kept at body temperature in an apparatus similar to that described by one of us (J. H. L.) (23). The gall bladder was then thoroughly rinsed free of bile by running Locke's solution through burette *A* into the lumen and out through side arm *C*, the arrangement of the cannula being such that the incoming stream enters the fundus of the viscus, *f*, and the outgoing stream leaves at the neck, *n*. During washing the apparatus was manipulated until all air in the gall bladder and in the glass parts below the stopcocks had been driven out. When washing was completed, i.e., the outgoing fluid had become perfectly clear,

[1] The bile contained in the animal's gall bladder was usually 1 to 2 cc. per kilogram of its body weight.

stopcock *A* was also closed and the entire content of the gall bladder siphoned off through side-arm *C*. After closing stopcock *C*, a given amount of Locke's solution—usually equivalent in volume to the bile removed from the viscus—was slowly introduced into the gall bladder from burette *A*.

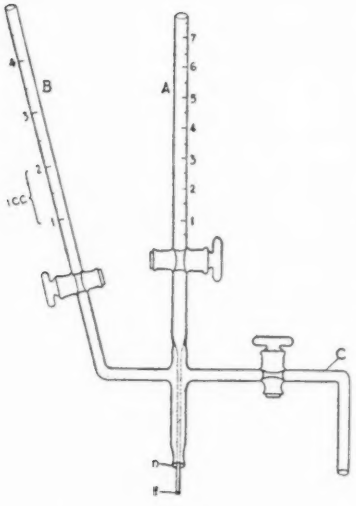


Fig. 1

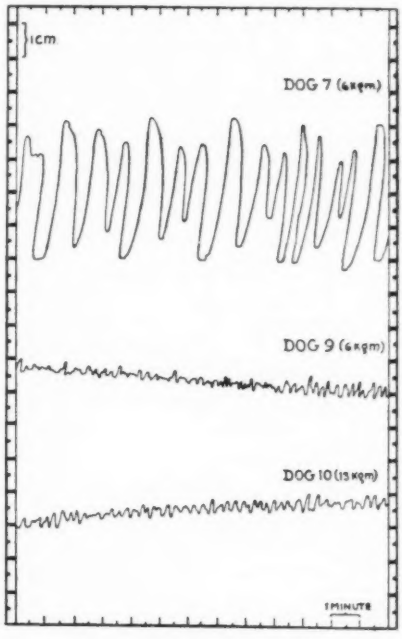


Fig. 2

**Fig. 1.** A cannula permitting accurate estimation of the fluid content of the isolated whole gall bladder and also the measuring of the movements of the vesicle in terms of hydrostatic pressure.

**Fig. 2.** Spontaneous contractions of the isolated whole gall bladder of the dog. The upper curve (dog 7) shows spontaneous rhythmic contractions as high as 46 mm. The middle curve (dog 9) shows spontaneous rhythmic contractions, and a slight decrease in tonus. The lower curve (dog 10) shows rhythmic contractions and a tonic contraction which reached a height of about 12 mm. within half an hour.

Before the last 1 or 2 cc. of fluid were introduced, burette *B* was attached to a Marey tambour and the stopcock of this burette opened. The final 1 or 2 cc. were then allowed to enter the viscus. This interrupted filling had two purposes: first, it put the diaphragm of the tambour under slight tension, and second, it usually assured that the fluid level was at the zero mark or above. Displacement of 0.5 cc. of fluid in burette *B* corresponded

on an average to 1 cm. level change of the writing hand, and represented in our experiments a power to displace a column of 0.5 cc. of Locke's solution against a hydrostatic pressure of about 12 cm. of the same fluid plus the resistance offered by the writing tambour.

*Spontaneous movements.* Spontaneous movements usually commenced soon after proper adjustments were made. These movements (fig. 2) were of two types and due to, *a*, spontaneous rhythmic contractions, and to *b*, tonic contractions.

*a.* The spontaneous rhythmic contractions showed a variety in height, duration and regularity. They usually changed in the course of the

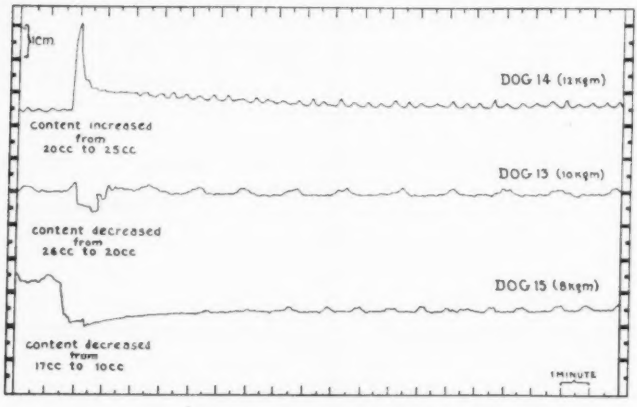


Fig. 3. The effect of change in volume upon the spontaneous movements of the isolated whole gall bladder of the dog. These curves illustrate that when the fluid content of the gall bladder was increased or decreased, adjustment of tonus soon occurred and the pressure in the viscus eventually returned to about its previous level and the curve gradually resumed its former shape.

experiment, although it appeared at first as if each gall bladder had its individual rhythm. Refreshing the bath was apt to modify the rhythm. The spontaneous rhythmic contractions were usually 2 to 30 mm. high—equivalent to the displacement of about 0.1 to 1.5 cc. of Locke's solution against a hydrostatic pressure of about 12 cm. of the same fluid plus the resistance offered by the writing tambour (1 to 4.3 cm. of hydrostatic pressure)—and occurred at a rate of about 1 to 3 per minute. The highest recorded was 46 mm.—equivalent to the displacement of about 2.3 cc. of Locke's solution under the same conditions. As many as 6 contractions per minute of 1 to 4 mm. height were occasionally recorded.

*b.* The tonic contractions which occurred spontaneously were rare and rather slow, reaching no significant height within the span of one hour.

Occasionally, however, an increase or decrease of about 10 mm. occurred within half an hour.

The effect of the following upon the spontaneous movements of the dog's isolated whole gall bladder was investigated: change in volume,

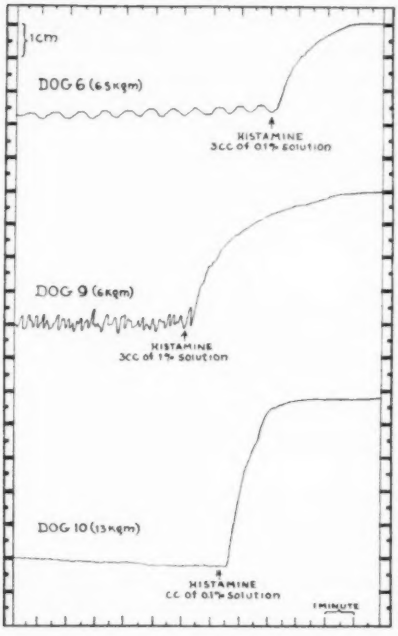


Fig. 4

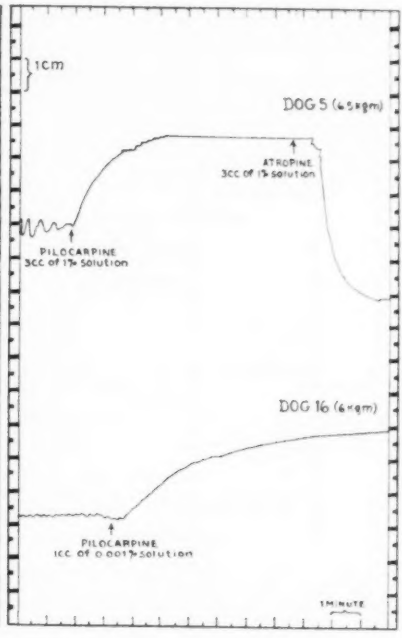


Fig. 5

Fig. 4. The effect of histamine upon the spontaneous movements of the isolated whole gall bladder of the dog. The rhythmic contractions ceased, the tonic contractions ranged between 26 to 50 mm.

Fig. 5. The effect of pilocarpine upon the spontaneous movements of the isolated whole gall bladder of the dog. The rhythmic contractions ceased, the tonus rose. The upper curve (dog 5) shows also the effect of atropine upon the tonus produced by pilocarpine.

histamine, pilocarpine, atropine, epinephrine, Koehler's extract of the suprarenal cortex (24), pituitrin, and cholecystokinin [Ivy-Oldberg] (10).

*The effect of change in volume upon the spontaneous movements.* When the fluid content of the gall bladder was increased or decreased, the change in pressure was registered, but soon adjustment of tonus occurred and the pressure in the viscus eventually returned to about its previous level and the curve gradually resumed its former shape (fig. 3).

*The effect of histamine (a), pilocarpine (b) and atropine (c) upon the spontaneous movements.* The effect of these drugs upon the dog's isolated whole gall bladder was about the same as it is on smooth muscle in general.

(*a*) *Histamine* (fig. 4), in doses of 2 or 3 cc. of a 0.1 per cent solution and 3 cc. of a 1 per cent solution, added to the bath of about 250 cc. of Locke's solution, caused a sudden rise in tonus and cessation of the rhythmic contractions. Usually within 5 minutes a constant level was reached. The elevation ranged between 26 and 50 mm., equivalent approximately to the displacement of 1.3 and 2.5 cc. respectively of Locke's solution against

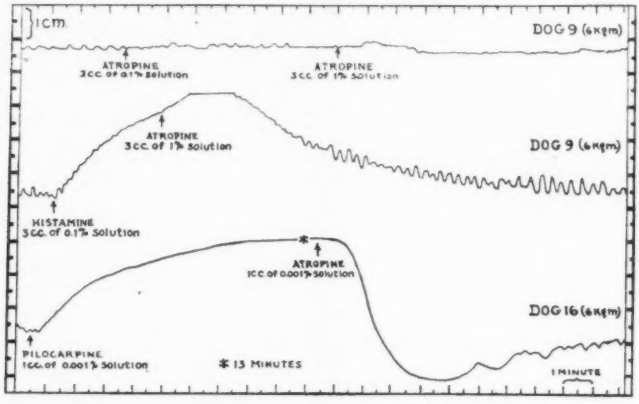


Fig. 6. The effect of atropine upon the spontaneous movements and upon the contractions produced by histamine and pilocarpine of the isolated whole gall bladder of the dog. The upper curve (dog 9) shows that atropine had no effect on the tonus but disturbed slightly the rhythmic contractions. The middle curve (dog 9) shows the effect of atropine, upon the tonic contraction produced by histamine. The lower curve (dog 16) shows the effect of atropine upon the tonic contraction produced by pilocarpine after the tonus had been maintained at about the same level for 13 minutes.

a hydrostatic pressure of about 12 cm. of the same fluid and against the resistance offered by the writing tambour (4.15 to 5.06 cm. of hydrostatic pressure).

(*b*) *Pilocarpine* (fig. 5), in doses of 1 cc. of a 0.001 per cent and 3 cc. of a 1 per cent solution added to the bath of about 250 cc. of Locke's solution, had an effect similar to that of histamine, i.e., a sudden rise in tonus and cessation of the rhythmic contractions. The elevation ranged between 26 and 30 mm., corresponding to the displacement of about 1.3 to 1.5 cc. of Locke's solution against a hydrostatic pressure of about 12 cm. of the same fluid and against the resistance offered by the writing tambour (4.15 to 4.3 cm. of hydrostatic pressure).

(c) *Atropine*, 3 cc. of an 0.1 per cent solution added to the bath of about 250 cc. of Locke's solution, had no effect on the tonus but disturbed slightly the rhythmic movements. Another dose—3 cc. of a 1 per cent solution—almost abolished the rhythmic movements, but had practically no effect on the tonus. Atropine decreased the tonus produced by histamine or pilocarpine (fig. 6).

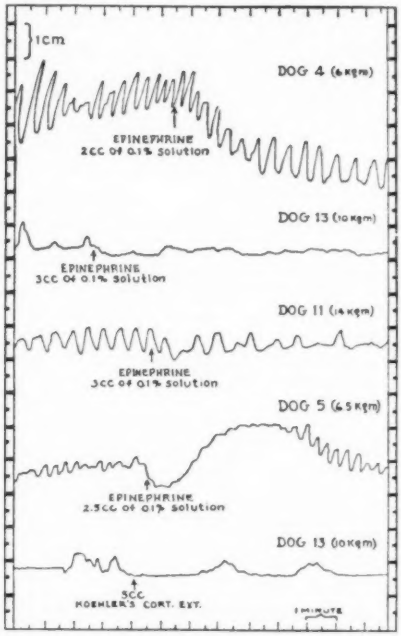


Fig. 7. The effect of epinephrine and Koehler's extract of the suprarenal cortex upon the spontaneous movements of the isolated whole gall bladder of the dog. Adrin [Mulford] caused at one time (dog 4) a decrease in tonus leaving the rhythmic contractions unaltered. At another time (dog 13 and dog 11) it had no effect on the tonus but disturbed the rhythmic contractions. Again (dog 5) after some decrease in tonus (6 mm.) an elevation (18 mm.) occurred. Koehler's extract of the suprarenal cortex (dog 13) had no influence upon the spontaneous movements.

*The effect of epinephrine and Koehler's extract of the suprarenal cortex upon the spontaneous movements.* Adrin [Mulford] (fig. 7): 2 cc. of a 1:1000 solution added to the bath of about 250 cc. of Locke's solution, caused at one time a decrease in tonus leaving the rhythmic contractions unaltered. This decrease in tonus was 26 mm., equivalent to the hydrostatic pressure of about 1.3 cc. of Locke's solution. At another time, 3 cc. of a 1:1000

solution, had no effect on the tonus but disturbed the rhythmic contractions. Again after some decrease in tonus (2 to 6 mm.) an elevation occurred ranging between 6 and 18 mm., equivalent to the displacement of about 0.3 to 0.9 cc. of Locke's solution against a hydrostatic pressure of about 12 cm. of the same fluid plus the resistance offered by the writing tambour (1.8 to 2.95 cm. of hydrostatic pressure).

Koehler's extract of the suprarenal cortex[2]: 5 cc. added to the bath of about 250 cc. of Locke's solution, had no influence upon the spontaneous movements of the dog's isolated whole gall bladder (fig. 7).

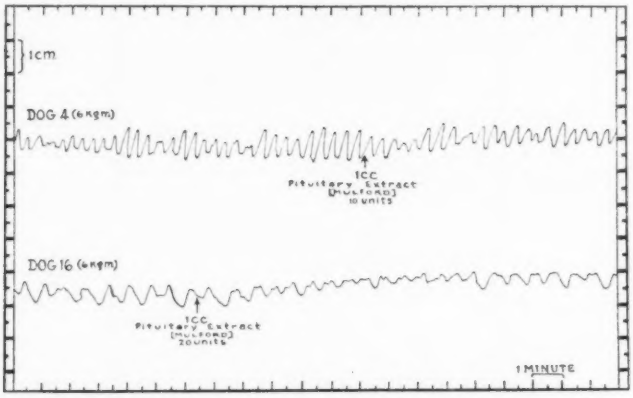


Fig. 8. The effect of pituitrin upon the spontaneous movements of the isolated whole gall bladder of the dog. Pituitary extract [Mulford] had no appreciable effect on either the tonus or the rhythmic contractions.

*The effect of pituitrin upon the spontaneous movements.* Pituitary extract [Mulford], in doses of 1 cc. containing 10 units or 20 units added to the bath of about 250 cc. of Locke's solution, had no appreciable effect on either the tonus or the rhythmic contractions (fig. 8).

*The effect of cholecystokinin [Ivy-Oldberg] upon the spontaneous movements.* Ivy and Oldberg's cholecystokinin[3], in doses of 3 to 8 cc. added to the bath of about 250 cc. of Locke's solution, had no effect whatsoever on the dog's isolated whole gall bladder (fig. 9) (25).

Discussion. Up to date, there is no consensus of opinion regarding the purpose and functions of the biliary vesicle. The discussion centers about

[2] The preparation was kindly supplied by Dr. Alfred E. Koehler of the Laskar Foundation for Medical Research and the Department of Medicine, University of Chicago.

[3] The preparation was kindly supplied by Dr. A. C. Ivy of the Department of Physiology, Northwestern University.

the fate of the bile entering the viscus. Does the bile enter the gall bladder to be stored there and expelled in time, or does it enter the viscus to be resorbed *in toto* by the gall bladder mucosa? In other words, does the bile that once has entered the gall bladder leave it again through the cystic duct, or is the bile resorbed and the constituents then returned by the way of the veins and lymphatics into the liver and into the general circulation, respectively? In either event the rôle played by the muscular coat of the gall bladder is an important one. If the traditional concept is correct, the muscularis of the gall bladder must be the motor power that evacuates the viscus. If, on the other hand, this concept is wrong, the function of the muscular coat remains to be explained. That bile may and does leave the gall bladder occasionally in small quantities can not

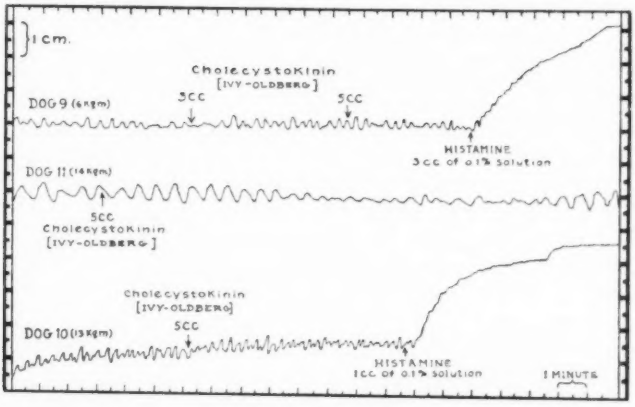


Fig. 9. The effect of Ivy and Oldberg's cholecystokinin upon the spontaneous movements of the isolated whole gall bladder of the dog. Ivy and Oldberg's cholecystokinin had no effect whatsoever upon the spontaneous movements.

be denied. It is perhaps also true that animals vary in this respect in as much as the anatomical arrangement of their biliary system differs. It has been shown by a number of investigators that the gall bladder can be made to expel some of its content through the cystic duct. Does that mean that the gall bladder regularly empties most of its content through the cystic duct or that its function is to do so?—Information on the contractile powers of the gall bladder ought to throw some light upon its motor mechanism and solve some of the debated problems.

The experiments of Lieb and McWhorter (16) and the subsequent investigations of Macht (5) and Ischiyama (17) established with fair accuracy the reactions of isolated strips of gall bladder wall to various stimuli. The few discrepancies reported by various observers at a later date are

obviously due to their more primitive method and less finished technique. It is only fair to state that these experiments do not furnish any data regarding the mechanism of the function or the contractile powers of the gall bladder and therefore such conclusions as have been drawn by Chiray and Pavel (26), Brugsch and Horsters (8), Erbsen and Damm (27), and by Kalk (22) are far from being warranted.

Ischiyama's experiments on the dog's isolated whole gall bladder suggested to us the possibility of expressing the movements in terms of hydrostatic pressure and thus obtaining an idea of the contractile powers of the biliary vesicle. We studied the reactions of the dog's isolated whole gall bladder with the aid of a specially constructed cannula that permitted accurate estimation, at any time, of the fluid content of the vesicle, the fluid expelled from the viscus by any given contraction, and the force exerted thereby.

As noted above, we observed spontaneous rhythmic contractions regularly and tonic contractions occasionally. The rhythmic contractions occurred at a rate of one to three or more per minute and represented a power to displace a column of about 0.1 to 2.3 cc. of Locke's solution against a hydrostatic pressure of approximately 13 to 16.5 cm. of the same fluid. Tonic contractions rarely occurred spontaneously and rarely reached a higher level than that representing a power to displace more than 0.5 to 1 cc. of Locke's solution under the same conditions. Yet, of the two movements the tonic contractions are likely to play the more significant part in the process of filling or emptying of the gall bladder. When the fluid content of the viscus was increased or decreased, the change in pressure was registered, but soon adjustment of tonus occurred and the pressure in the viscus eventually returned to about its previous level and the curve gradually resumed its former shape. These observations strongly suggest that the function of the muscular coat of the gall bladder is that of effecting adjustment in size to the varying content rather than the emptying of the viscus (28).

The experiments with histamine, pilocarpine and atropine are of interest. Histamine and pilocarpine never failed to cause a sudden rise in tonus and cessation of the rhythmic contractions Atropine decreased the tonus produced by histamine or pilocarpine. The rise in tonus produced by histamine or pilocarpine, however, represented no more power than that necessary to displace a column of 2.5 cc. of Locke's solution against a hydrostatic pressure of approximately 17 cm. of the same fluid, i.e., slightly more than the recorded maximum spontaneous rhythmic contractions.

The effect of epinephrine, Koehler's extract of the suprarenal cortex and the effect of pituitrin need no further comment. It is interesting that cholecystokinin, which, according to Ivy and Oldberg, causes contraction

of the gall bladder in the intact or anesthetized dog, failed to influence the dog's isolated whole gall bladder.

## SUMMARY AND CONCLUSIONS

The reactions of the dog's isolated whole gall bladder were studied with the aim of obtaining data on the contractile powers of the biliary vesicle. This was accomplished with the aid of a specially constructed cannula that permitted accurate estimation, at any time, of the fluid content of the viscus and the measuring of the movements in terms of hydrostatic pressure.

Spontaneous rhythmic contractions were obtained regularly and tonic contractions occasionally. The rhythmic contractions represented a power to displace a column of about 0.1 to 2.3 cc. of Locke's solution against a hydrostatic pressure of approximately 13 to 16.5 cm. of the same fluid, and occurred at a rate of one to three or more per minute. Tonic contractions rarely occurred spontaneously and rarely reached a higher level than that representing a power to displace more than a column of 1 to 2 cc. of Locke's solution, under the same conditions.

The effect of histamine, pilocarpine and atropine was about the same as it is on smooth muscle in general. The effect of epinephrine was inconstant, affecting sometimes only the tonus or the rhythmic contractions and sometimes both. Pituitrin, cholecystokinin [Ivy-Oldberg] and Koehler's extract of the suprarenal cortex had no appreciable influence upon either the tonus or the rhythmic contractions.

When the fluid content of the gall bladder was increased or decreased, adjustment of tonus soon occurred and the pressure in the viscus eventually returned to about its previous level and the curve gradually resumed its former shape. This latter observation strongly suggests that the function of the muscular coat of the gall bladder is that of effecting adjustment in size to the varying content, rather than the emptying of the viscus.

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# THE RÔLE OF THE LUNG IN THE METABOLISM OF FAT

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In the last two decades papers have appeared in the literature dealing with the rôle of the lung in the metabolism of fat. From a teleologic point of view, the assumption is not without merit that the lung plays a vital part in the metabolism of fat, since a larger proportion of the fat is absorbed by the lacteals, and before it reaches the general circulation, it has to traverse the thoracic duct and the pulmonary circulation. Inasmuch as this phase of the subject does not appear to have been reviewed in the English literature a short bibliography is given of the literature dealing with this subject. We might state that the experiments which purport to involve the lung in the metabolism of fat have often been of such nature as to justify criticism, according to the physiologic standards of the present time. Much of the early work was done histologically; some was based on supposed differences in the amount of fat in the blood of the pulmonary artery and the pulmonary vein; other work was done by the perfusion of isolated organs. Many of the experiments were concerned with the formation of fat in autolysis observed at necropsy. In view of the significance that such a conclusion would have if it could be substantiated, we have undertaken a series of experiments to test the hypothesis that the lung plays an important part in the metabolism of fat.

METHOD OF EXPERIMENTS. Healthy dogs were used. In all experiments involving operative procedures the dogs were anesthetized with ether; and they were killed by bleeding under local anesthesia. Fatty acid in the tissues was estimated by Leathes' modification of Lieberman's method. Fat in the blood was estimated by Bloor's latest nephelometric method. Owing to the fact that the weather at that time was hot, the nephelometric readings were completed rapidly, as otherwise they were irregular. It was found that by using chilled distilled water as the diluting fluid the nephelometric readings were accurate if the comparisons were made within three to five minutes after acidification. We have every reason to believe that these analyses are accurate, as numerous controls on standard solutions have been checked. In several experiments the analyses of the fat in the blood were checked against Leathes' method, with

good agreement. Usually the estimations on blood and tissue were made in duplicate. The cholesterol fraction of the fat in the blood was not determined separately, since it was desirable to secure a comparison as a whole of the fat content of the blood before and after alimentation and proximal or distal to the lung.

In many experiments, histologic sections were made of the liver and the lung, and since Millot and Giberton found that the amount of fat in the tissues preserved in formalin decreased fairly rapidly, sections were cut within two or three days.

Results of experiments. In order to ascertain that the animals in our laboratory are in nowise exceptional as regards the fat content of tissues, two healthy dogs were fasted for eighteen hours, and then killed by bleeding. The fat in the lung of one dog was 2.5 and 2.6 per cent (duplicates), and in the liver 5.0 and 4.1 per cent (duplicates). The fat in the blood in the right side of the heart was 0.84 and 0.88 per cent (duplicates), and the fat in the blood obtained simultaneously from the femoral artery was 0.87 per cent. In the case of the second dog, the fat in the lung was 2.4 and 2.6 per cent (duplicates), and of the liver 7.1 and 7.7 per cent (duplicates). Since these data agree reasonably well with what is known, we concluded that nothing useful would be gained by investigating a larger series of normal controls.

The dogs were fasted for twenty-four hours. In some experiments 100 cc. of vegetable oil were given by stomach tube. In other experiments, a large meal of cream, whose fat content we analyzed, was given. When a measured amount of cream was given we determined the amount actually absorbed by removing the intestines at necropsy, washing out the contents thoroughly, and estimating the amount not absorbed. The difference gave the amount of fat absorbed. It might be added, parenthetically, that we have found cream sold to contain rather constantly 30 per cent butter-fat, and that the fatty acid, by weight, was 30 $\pm$ 2 per cent. However, in each experiment, this was estimated separately.

In one experiment, in order to duplicate some of the conditions prevailing in the experiments of previous workers, we injected intravenously a fine emulsion of fat, prepared by emulsifying olive oil with blood serum and sodium glychocholate. The particles of fat were shown to be not larger than 4 microns, microscopically.

In another experiment, in order to duplicate more accurately the natural conditions by which fat is absorbed, we injected, intravenously, a considerable quantity of citrated lymph obtained from a fistula of the thoracic duct of a dog fed on fat. The third dog was given 100 cc. of vegetable oil by stomach tube, and was killed four and a half hours later. The fatty acid in the lung was 3.2 per cent, and in the liver 3.9 and 4.0 per cent (duplicates). The results in two other dogs were similar. Following the

ingestion of a large quantity of vegetable oil by mouth, the fat in the lung was not significantly greater than that of the normal controls.

In view of the fact that in these experiments the fat administered was a vegetable oil, which possibly is more difficult to absorb than is an emulsion of animal fat, the experiments were changed so that the animal was given a measured amount of 30 per cent cream, and the amount that was actually absorbed was estimated by analyzing the intestinal contents at necropsy. In the fifth dog, who was given 160 grams of fat,[1] 67 grams were absorbed in four hours and three minutes. Since the animal weighed 4.5 kgm., this indicated an absorption of 15 grams for each kilogram of body weight. At necropsy, the fat in the lung and liver was 2.8 and 3.4 per cent, respectively. The fat in the blood before the administration of cream was 0.837 and 0.827 per cent (duplicates). At the end of the experiment, just before the animal was killed, the fat in the blood from the right side of the heart was 0.834 per cent and of the femoral artery 0.824 per cent. It is evident, from this experiment, that, although the dog absorbed a large amount of fat, the fat in the blood and in the lung was not appreciably increased at the end of the experiment. In the sixth dog, who was subjected to a similar regimen, the fat absorbed was 55.4 grams (7.3 gm. for each kilogram of body weight) in four and a half hours. This dog had been fasted for twenty-four hours. The fat removed from the blood just before the administration of cream was 0.788 per cent. The fat in the blood from the right side of the heart four and a half hours after the cream had been fed was 0.788 and 0.808 per cent (duplicates). The fat in the femoral artery was 0.744 per cent. At necropsy, the lungs, which weighed 70 grams had 5 per cent fat, and the liver, which weighed 220 grams had 4.8 per cent fat. This experiment is the only one of the series in which the amount of fat in the lung was greater than normal, following a meal of fat. Histologically, the tissue contained more fat than is normally present. Most of the areas showed a normal distribution of fat, but there were some patches with large masses of fat free in the alveoli, and partly filling them.

In the next experiment, specimens of the sartorius and the rectus femoris muscles were removed under ether anesthesia for estimation of the content of fat. The muscles were stripped of adherent fat and fascia so that only the fat in the muscle fibers was estimated. When the animal had recovered from the anesthetic, it drank 120 cc. of 30 per cent cream with relish, and at subsequent intervals, it drank 230 cc. with relish, so that, in all, the animal received 350 cc. of cream containing fatty acids, by analysis of 127 grams. The animal was anesthetized and blood was removed simultaneously from the portal vein, the right side of the heart, and the aorta. The content of fat was 0.783, 0.829 and 0.776 per cent respectively. The

[1] In each case the figures for fat are expressed as fatty acid.

animal was killed by bleeding, and specimens of the lung, liver and the muscles of the opposite side were removed for analysis. The fat in the sartorius muscle before feeding was 3.9 per cent and after feeding 3.6 per cent. The fat in the rectus femoris muscle before feeding was 7.8 per cent and after feeding 4.1 per cent. By estimating the fat remaining in the gastro-intestinal tract, it was calculated that the dog absorbed 43 grams of fat (5 gm. for each kilogram of body weight). Histologic sections of the lung, frozen and stained with Sudan III, did not show an increase in the content of fat. This was also true of the liver, except that there was more free fat in the bile duct. In the specimens of muscle there was no difference histologically in the amount of fat in the specimens before and after fat had been fed.

The foregoing experiment confirms those made previously. Moreover, we have been unable to detect an increase of fat in corresponding muscles of the opposite sides, following the absorption of a quantity of fat. Although the content of glycogen of corresponding muscles on opposite sides of the body may be approximately equal, this has not been shown to be true for fat. A number of other experiments of similar nature were performed with identical results.

In view of the fact that the absorption of fat, although considerable in these experiments, was not sufficient to provoke marked lipemia, the plan of the experiments was changed so that specimens of lung and liver were removed under ether anesthesia for the analysis of fat and jejunostomy was performed. When the animal had recovered from the anesthetic, large quantities of cream were introduced through the stoma. In one case, the cream was first incubated with bile and pancreatic juice for one hour. The results were not changed. We might state that this technic is not an ideal one, since the amount of fat absorbed by the animals was actually less, probably in consequence of the reaction to the operation. In one experiment, a dog weighing 6.7 kgm. in the post-absorptive condition, was given 65 cc. of citrated chyle, obtained from the thoracic duct of a dog fed on fat; the amount of fat thus injected was found by analysis to be 2.9 grams. Blood drawn from the jugular vein before the injection contained 0.864 per cent of fat. Blood drawn immediately after the injection (twenty-five minutes after its beginning) contained 1.55 per cent of fat. Forty minutes later, blood drawn from the femoral artery contained 0.86 and 0.87 per cent of fat (duplicates). Blood drawn from the right side of the heart contained 0.83 and 0.76 per cent of fat (duplicates). The animal was then killed. The lung contained 2.9 and 3.1 per cent of fat (duplicates); the liver contained 3.8 and 3.9 per cent (duplicates).

Our final experiment has an outstanding significance, in view of reports in the literature concerning deductions about the lipolytic functions of the lung. These deductions are based on the results of injecting emulsions

of fat intravenously. A dog, weighing 3.7 kgm., was etherized and the left lobe of the liver and the lower right lobe of the lung were removed for estimation of the content of fat. The right femoral vein was then exposed for injection of the fat. When the animal had recovered from the immediate effects of the operation, an especially prepared emulsion of fat was injected, in which the particles were not more than 4 microns in diameter. To 1 cc. of the emulsion 0.48 gram of fatty acid was added, and 28 cc. of the emulsion were injected over a period of twenty minutes. The blood from the right side of the heart, ten minutes after the injection was completed, contained 2.86 per cent fat, and blood from the left side of the heart, removed one minute later, contained 2.55 per cent of fat. The blood was intensely lipemic. The animal was killed by bleeding under local anesthesia. The lung weighed 39 grams and the liver weighed 90 grams. Before the injection, the fat in the lung and in the liver was 2.8 and 3.5 per cent, respectively, and after the injection it was 18.0 and 19.0 per cent, respectively. The liver had held back 5 grams of fat, and the lung had held back 6 grams of fat. Histologically, specimens of liver were markedly infiltrated with fat. The sinusoids were filled with fat, and the hepatic and Kupffer cells were crowded with fat; even the portal and central veins had free fat inside the lumen. The bile ducts were well filled with fat. Large masses of fat were present in the capillaries of the lung and in the alveolar cells, and free fat was present in the alveoli and in the bronchi.

This experiment is extremely significant in that although an emulsion of fat was injected whose particles were theoretically too small to give rise to fat embolism, the histologic and chemical picture was undoubtedly best explained on the basis that the injected fat had acted embolically. Inasmuch as nearly all of the experiments which are reported in the literature, purporting to involve the lung in the metabolism of fat, have been based on the injection of fat emulsion, we would suggest that such results do not portray accurately what actually occurs in the normal course of the digestion and assimilation of fat.

COMMENT. A number of observations regarding the rôle of the lung in the metabolism of fat may be culled from the literature. First, histologically the lung manifests under normal conditions large globules of fat, which appear similar to the aggregation of fat encountered in fat embolism (Gilbert and Jomier, 1905). Second, the content of fat in the lung is much increased following the intravenous injection of oil and of highly emulsified fat (Köszeg, 1924; Gilbert and Jomier, 1924; Busquet and Vischniac, 1921; Scheffer, 1927). Third, the content of fat of the lung is either not increased when the organism is fed with large quantities of fat (Terroine and Weille) or is increased (Roger and Binet, 1921, 1922), and fourth, the blood in the pulmonary artery of a dog fed on fat is 10 per cent richer

in fat than is the blood in the femoral artery (Gilbert and Jomier; Roger and Binet). The last suggestion does not seem likely, since it would appear that in the course of a few minutes, the circulating blood would be clear of fat. Assuming that the circulation of the blood in a dog takes about sixteen seconds for one cycle, it becomes apparent that the foregoing conclusion is improbable.

It may, therefore, be considered that the evidence that the lung has a special rôle in the metabolism of fat may depend on numerous observations that large quantities of fat accumulate in the lungs when finely divided emulsions of fat are injected intravenously. It has been assumed that, since the particles of fat are smaller than the erythrocytes, the fat would not be trapped in the lung embolically. The assumption is not well founded, since in our experiments, after we had injected a very fine emulsion of fat, in which the particles were no greater than 4 microns in diameter, there was an enormous increase of fat in the lungs, whereas an increase could not be demonstrated when the animals were fed large quantities of fat by mouth. We have not been able to demonstrate any significant increase of fat in the lung following the absorption of as much as 8 grams of butter-fat for each kilogram of body weight over a course of four hours. Our experiments have shown that the blood of the pulmonary artery contained more fat than blood from the femoral artery only when an emulsion of fat was injected intravenously. Otherwise, the amounts represented excellent duplicates. It would appear that the particles of the fat emulsion become fused to larger particles in the blood stream and give rise to fat embolism of the lungs.

## SUMMARY

A number of experiments were performed in an attempt to evaluate the rôle of the lung in the metabolism of fat; the analysis for fat in the blood was carried out by Bloor's method and for fat in the blood and tissues by Leathe's method. Specimens of lung were removed for analysis under aseptic conditions, and the experiments were performed after the animal had fully recovered. The fat administered was a measured amount of 30 per cent cream, which the animals drank voluntarily. In some experiments, jejunostomy was performed aseptically at the time the specimen of lung was removed, and the fat was administered through the stoma after the animal had recovered from the immediate effects of the operation. To ascertain if the fat was actually absorbed, the amount of fat remaining in the bowel was estimated at necropsy. It was found that the fat in the lung had not increased, nor was there any appreciable difference in the amount of fat in the blood proximal and distal to the lung, as has been stated by various previous workers. The results were checked histologically. It was not possible to demonstrate that the content of fat in the lung in-

creased following the intravenous injection of citrated chyle obtained from a dog fed on fat. However, in one experiment, in which a highly emulsified preparation of fat was injected intravenously, there was a huge increase of fat in the lung, and a definite diminution of fat in the blood from the femoral artery, as compared with blood removed from the right ventricle.

The results of our experiments have not furnished evidence for the supposition that the lung plays an especial rôle in the metabolism of fat.

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# THE REGULATION OF RESPIRATION

## XXXIV. The Carbon Dioxide Content of the Intact Brain of the Frog in Relation to Changes of Oxidations

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With increasing emphasis placed on the metabolism of the respiratory center as an important factor in determining the respiratory activity of the animal (Gesell, 1929), it becomes essential that more be known of the acid base equilibria and buffering capacities of the center under various respiratory conditions, particularly those in which lactic acid content is altered and as a consequence of which hydrogen ion concentration is increased or diminished.

It has been shown that lactic acid accumulates in the isolated brain under anaerobic conditions at a rate considerably in excess of the production of carbon dioxide during normal oxidations (McGinty and Gesell, 1925). In more recent experiments it has been shown that under conditions of normal oxidations in brain tissue lactic acid is absorbed from the arterial blood during its passage through brain capillaries and with impairment of oxidations during cyanide poisoning or by diminishing oxygen supply to the cells, there occurs excessive lactic acid production in the brain with an outward diffusion into the blood stream (McGinty, 1928–29). More recently Kleinschmidt (1929) has shown that reduced oxidations in the intact brain of the dog following hemorrhage or injection of sodium cyanide result in a marked reduction in total carbon dioxide content and of buffering capacity of brain tissue. It is, therefore, possible that the hyperpnea which accompanies impaired oxidations in the intact animal may be associated with increased acidity of the respiratory neurones despite the fact that hyperpnea lowers the carbon dioxide pressure and the hydrogen ion concentration of the blood below the normal values. To determine approximately the resultant of the opposing effects of the decrease in carbon dioxide pressure on the one hand and increase in lactic acid and decrease in buffer base on the other hand it seemed desirable to establish carbon dioxide dissociation curves of the frog's brain during normal and subnormal oxidations. With the application of the Henderson-Hassel-

balch (1919) equation to tissues as employed by Wastl and Seliškar (1925) and Fenn (1928) such an analysis was attempted.

METHOD. Intact frogs (R. pipiens) previously kept for at least one week in water at a temperature of 11° to 13°C. were exposed in equilibration chambers to varying carbon dioxide tensions in room air, in high oxygen gaseous mixtures, in low oxygen gaseous mixtures, and in room air after subcutaneous administration of sodium cyanide. After exposure to the gaseous mixtures at approximately 23° for periods ranging from 45 minutes to 2 hours the frogs were transferred quickly to a glass top dissecting box containing the same gaseous mixture. The hands and forearm of the operator projected into the dissecting chamber through air tight rubber cuffs. The frog was decapitated and the entire brain and medulla dissected out posteriorly considerable care being taken to avoid rupture of the endolymphatic sac which practically encircles the brain below the optic lobes and which contains a watery suspension of crystalline $CaCO_3$. Very fluctuating values were obtained unless these precautions were observed. The brain was transferred to a small tared glass capsule containing sufficient glycerol to cover the tissue. The entire procedure took no longer than 2 minutes. After weighing, the brain was introduced into the apparatus described by Fenn (1928) and total carbon dioxide determined by the $Ba(OH)_2$ electrical conductivity method. The conductivity cell constant was checked from time to time and control carbon dioxide determinations on standardized $NaHCO_3$ were carried out at frequent intervals.

RESULTS AND DISCUSSION. The results are plotted in figure 1, each point on the curve representing an average of six to twenty determinations on as many frogs. With administration of carbon dioxide in room air and in high oxygen mixtures, equilibrium between the brain carbon dioxide and respired gas was attained in approximately 30 minutes. This was true even at the higher concentrations of carbon dioxide. To be certain of complete equilibrium the frogs were exposed uniformly for 45 minutes in the carbon dioxide room air mixtures and for one hour in the carbon dioxide high oxygen mixtures, the gas in the chambers being continuously renewed during this period.

During preliminary work it became evident that reduction in percentage of oxygen in an oxygen-nitrogen mixture was without demonstrable effect on the carbon dioxide level in the brain until a concentration below 9 per cent was reached (see table 1). Reduction of oxygen content below this level depressed tissue carbon dioxide in proportion to the extent of reduction in oxygen concentration. Likewise it was found that at higher tensions of carbon dioxide in a carbon dioxide low oxygen mixture, reduction of the oxygen level to 3.0 to 4.0 per cent was the limit that the frog could tolerate and in subsequent work the oxygen tension was maintained at approximately this point. Even at 3 to 4 per cent oxygen the

frog began to fail with continued exposure and with additional carbon dioxide pressures above 125 mm., tissue carbon dioxide diminished with reduction in oxidations so that under these conditions equilibrium was never reached. Since duration of equilibration as well as concentration of oxygen in the gaseous mixtures contributed toward the net impairment of oxidation a uniform period of 2 hours of exposure in the chambers was taken with carbon dioxide low oxygen mixtures.

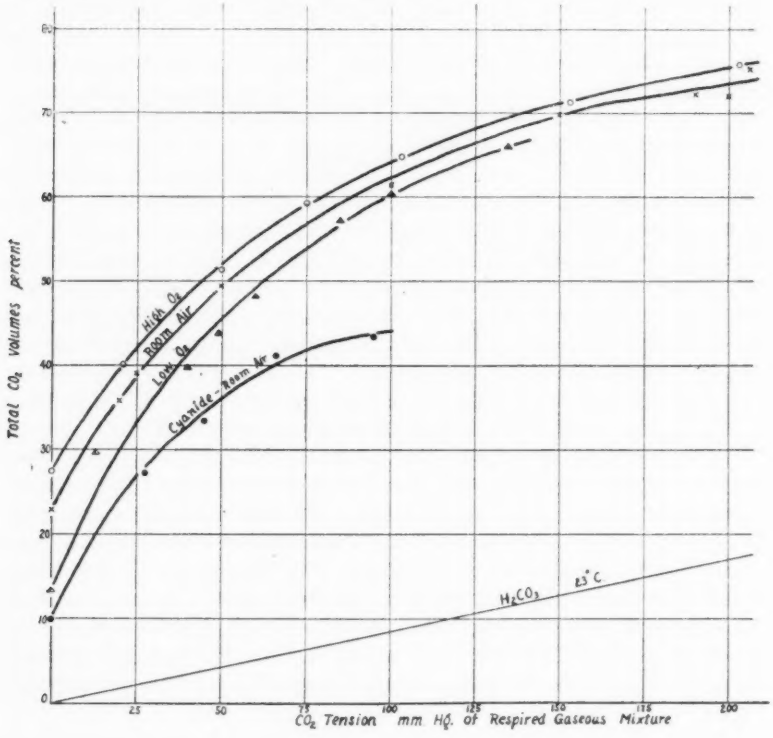


Fig. 1

In a similar manner the amount of injected NaCN necessary to produce a maximal reduction in tissue oxidations without onset of convulsions was found to be 1 cc. of 0.005 N. NaCN per 30 grams body weight. This was introduced with a hypodermic syringe into the dorsal lymph space and the frogs were placed at once into the chamber containing the experimental gaseous mixture for a period of one hour. Under these conditions the maximal carbon dioxide tension that the frog could tolerate was never more

than 100 mm. It could not be observed conclusively whether respiratory movements differed from those in room air on administration of any of the experimental gaseous mixtures.

The average total carbon dioxide content of the normal frog brain when breathing room air was 22.8 volumes per cent. In an atmosphere of pure oxygen the carbon dioxide content of the brain increased to 27.4 volumes per cent and in low oxygen (3.0 to 4.0 per cent) the carbon dioxide content was reduced to 13.3 volumes per cent. With injections of cyanide and breathing room air the carbon dioxide content of the brain fell to an average of 9.9 volumes per cent at the end of one hour. From these figures and the general level of the curves it is quite evident that lowered alveolar oxygen and injection of sodium cyanide increase the formation and accumulation of fixed acids in the brain with an associated decrease in bicarbonate buffer base and that increased alveolar oxygen with its reverse effects on oxidations increases the bicarbonate buffer base.

TABLE 1

| NUMBER OF EXPERIMENTS | GASEOUS MIXTURE; PER CENT OXYGEN IN NITROGEN | TOTAL $CO_2$ |
|---|---|---|
| | | *vols. per cent* |
| 20 | 20.9 (room air) | 22.8 |
| 12 | 9.0 | 20.2 |
| 6 | 5.4 | 16.2 |
| 14 | 3.5 (average) | 13.3 |
| 6 | 2.0 | 6.5 |

* Period of equilibration approximately 90 minutes.

In figure 2 the curves have been corrected for dissolved carbon dioxide (assuming the brain substance to be 80 per cent water) and replotted so that the ordinates on this diagram represent only carbon dioxide in combined form. With the use of the Henderson-Hasselbalch equation (Haggard and Henderson, 1919) and a pK value of 6.17, pH diagonals have been added to the diagram. In treating the curves as true carbon dioxide dissociation curves inaccuracies are apparent, most striking at the lower carbon dioxide pressures. Obviously the carbon dioxide pressure in the tissues can never be zero but is under normal conditions as estimated by Fenn and by Wastl and Seliškar from 10 to 25 mm. Hg. No effort has been made to correct or use the left hand portions of the curves but it has been assumed that with increasing carbon dioxide pressure in the equilibration chamber, the curves approximate more and more closely the true carbon dioxide dissociation curves and that after 25 mm. Hg pressure has been reached the curves suffice for our approximations. The method to be sure is not precise. Yet the bulk of the brain and the slow rate of dif-

fusion of oxygen and carbon dioxide through the interior cells preclude the equilibration of the isolated brain.

It should, therefore, be kept in mind that the absolute position of the pH diagonals is somewhat arbitrary and may be used only in a relative manner to indicate the comparative buffering values of the brain with change in alveolar oxygen pressures and after the introduction of sodium cyanide. At pH of 7.2 in the brain the carbon dioxide tension would be approximately 33 mm. At the same pH the carbon dioxide tension with the high oxygen carbon dioxide mixture would be 36 mm. In a low oxy-

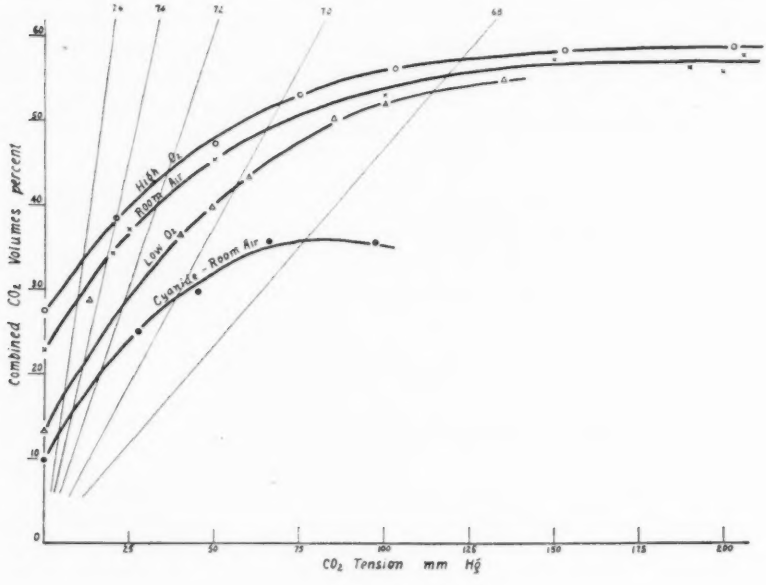


Fig. 2

gen carbon dioxide mixture and in a room air carbon dioxide mixture during cyanide administration at brain pH of 7.2 the carbon dioxide pressures would be 23 and 16 mm. respectively. The favorable effects of high oxygen and the adverse effects of low alveolar oxygen and of cyanide on the carbon dioxide tensions at the same hydrogen ion concentration in the brain are evident. In a similar way it may be observed that at an alveolar carbon dioxide tension of 25 mm. the pH of the brain during high oxygen administration is approximately 7.33 while in room air at the same carbon dioxide pressure the pH is 7.28. With low oxygen and after cyanide injection at 25 mm. carbon dioxide pressure the pH is 7.19 and 7.10 respec-

tively. Evidence of a tendency toward increased tissue acidity with the diminished oxidations of low oxygen and cyanide administration and decreased acidity with high oxygen thus supports the general theory that altered metabolism within the cells, and changing acidity of central nerve tissue may assume a major rôle in control of certain vital activities, e.g., pulmonary ventilation and circulation of the blood.

In figure 3, the carbon dioxide curve of intact frog brain in room air is plotted along with the carbon dioxide dissociation curves of frog's blood

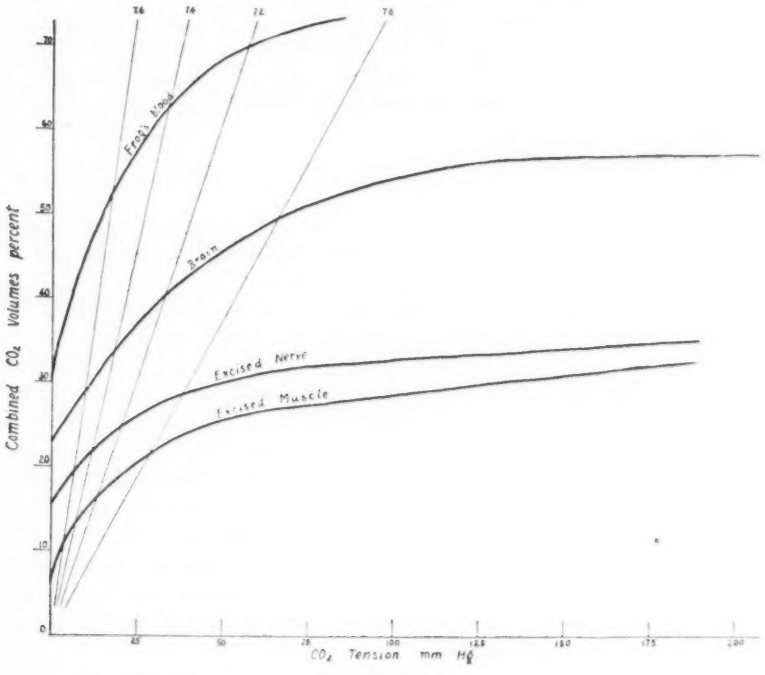


Fig. 3

(Wastl and Seliškar, 1925) and of excised frog's nerve and muscle (Fenn, 1928). Diagonals showing pH are added.

Comparing the carbon dioxide curves of frog's brain with carbon dioxide dissociation curves of blood and excised tissues, with the reservations already mentioned it appears that the pH of the blood is considerably higher at similar alveolar carbon dioxide pressures than the pH of the brain, agreeing with the generally accepted view that the acidity of tissue cells is greater than that of the blood. The acidity of brain tissue, however, is

less than that of nerve or muscle under similar conditions. Proceeding along an isohydric diagonal it is observed that the buffers of the blood are much more effective than those of the brain but that the buffering capacity of brain substance is superior to that of nerve or muscle.

The comparative buffering capacity of blood, brain, nerve and muscle most probably has a significance which as yet is not clear. The high buffering capacity of the brain must undoubtedly act as an effective protective mechanism against the exceedingly rapid development of lactic acid during anaerobic conditions as was demonstrated by McGinty and Gesell (1925). Even with partial occlusion of the cranial arteries, normal lactic acid absorption gives way to excessive formation and outward diffusion into the blood stream (McGinty, 1929). Whether this defeats a sensitive regulatory reaction to changes in hydrogen ion concentration, however, is another question.

## SUMMARY

Intact living frogs were equilibrated for periods of 45 minutes to 2 hours in gaseous mixtures containing varying amounts of carbon dioxide in room air, in high and low oxygen gaseous mixtures and in room air during simultaneous administration of sodium cyanide in the dorsal lymph sac.

At the end of the equilibration period, the frog brain was removed quickly, with due precaution to prevent gas exchange, and analysed for total carbon dioxide by the $Ba(OH)_2$ electrical conductivity method.

The averages of values thus obtained were plotted on ordinates with carbon dioxide tensions on absissae. The total carbon dioxide content of the brain of a frog kept in a high oxygen gaseous mixture, in room air, in a low oxygen mixture and in room air during cyanide poisoning were 27.4, 22.8, 13.3 and 9.9 volumes per cent respectively. When carbon dioxide was added to these gaseous mixtures the same relative positions of the curves held true. These results indicate an increased buffering capacity of the brain when oxidations are improved and a decreased buffering capacity when oxidations are impaired.

Applying the Henderson-Hasselbalch equation it is computed that for a carbon dioxide pressure of 25 mm. Hg the pH of the brain in the order of the conditions named would be approximately 7.33, 7.28, 7.19 and 7.10. Conversely, for a pH of 7.2 the carbon dioxide pressure would be approximately 36, 33, 23 and 16 mm. Hg. It is, therefore, suggested that the decreased carbon dioxide pressure and hydrogen ion concentration of the blood commonly associated with temporarily impaired oxidations may be accompanied by an increased acidity of the brain.

When the curves of these experiments are compared with carbon dioxide dissociation curves of frog's blood or of those of excised muscle and nerve

obtained with other methods it appears that frog's brain is better buffered than frog's muscle or nerve but less well buffered than frog's blood.

The significance of the relative buffering capacity of brain compared to that of blood, muscle and nerve is discussed.

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# PHYSIOLOGICAL VARIATIONS OF THE CARDIAC OUTPUT OF MAN

## VIII. The Constancy of the Cardiac Output from Day to Day Throughout the Year[1]

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The purpose of the present investigation was to determine the degree of constancy of the cardiac output from day to day, and to discover the factors which cause its variation. It is important to know whether the cardiac output, like certain other physiological functions, is relatively constant under fixed conditions or whether it is subject to daily or seasonal fluctuations. The solution of this problem is of particular importance where it is necessary to carry out determinations over a long period of time. Obviously, it is impossible to evaluate data obtained in such a series, unless one knows the daily changes which would be expected in normal individuals.

Of previous investigators who have attempted to evaluate the daily variation of the cardiac output, Lindhard (1913) concluded that the cardiac output undergoes a seasonal variation. Collett and Liljestrand (1924) in their study of a female subject over several menstrual cycles concluded that her cardiac output underwent a definite catamenial cycle. Burwell and Robinson (1928) present data on one individual, made on 11 different days, over a period of 5 years. They concluded that "the output of the heart remained relatively unchanged throughout that period." Three of their thirteen values, however, range from 4.70 to 5.24 liters instead of 3.63 to 3.96 which is the range of the other determinations.

Methods. The determinations recorded in the present paper were made over a period of a year and a half, from June, 1928, to January, 1930. The earlier determinations—those from June, 1928, to December, 1928—were made by the use of nitrous oxide (Marshall and Grollman, 1928). The results obtained by the use of this gas are less accurate than those obtained by the use of acetylene (Grollman, 1929a); the latter gas was, therefore, used exclusively in all experiments quoted after December 25, 1928. The

[1] A preliminary account of this work was presented before the XIIIth International Physiological Congress, Boston, 1929.

determinations by the nitrous oxide method were done in duplicate and the average values are quoted. They should not be in error greater than ±10 per cent. The results by the acetylene method could easily be duplicated in successive experiments to within ±5 per cent and this may be considered as the accuracy of all the results recorded after December 25, 1928.

In order to obtain truly basal conditions and to avoid the vitiating influences of factors, which might affect the cardiac output, stringent experimental conditions were observed in obtaining the values recorded in table 1. To attain ideal basal conditions, the experiments were performed soon after the subject's spontaneous awakening in the morning. Within five or ten minutes after awaking, the subject began breathing into a Douglas bag for the purpose of determining the oxygen consumption. The pulse rate was then determined and the experiment concluded by the determination of the cardiac output. The experiment was so arranged that it was not necessary for the subject to move or be disturbed from the recumbent position in bed. The experiments were performed at the home of the subject.

The subjects studied in the present investigation were healthy, young individuals of good physique and complacent temperament. On the night preceding any determination they always had a satisfactory night's rest. They had not partaken of food or drink for at least twelve hours before the experiment, and thus any effects of the ingestion of food or liquids (Grollman, 1929b, c) were avoided. Psychic factors or disturbances (Grollman, 1929d) were also avoided. In order to obviate the effect of temperature changes, the room in which the experiments were performed seldom varied throughout the year beyond 20 to 23°.

With the above described precautions the results obtained should be truly basal, in the strict sense of the word, and should represent the degree of variation of the cardiac output of the human subject under relatively constant conditions.

RESULTS. *The variation of the cardiac output of a male subject from day to day.* A series of determinations made as described above on a male subject, *A. G.*, is recorded in table 1. The table omits results obtained from May 25, 1929, to August 30, 1929, when the subject was engaged in an expedition to Pike's Peak. The results obtained during this time were reported in a paper dealing with the effect of high altitudes on the cardiac output (Grollman, 1930). Comparison of the results recorded as table 8 of the above mentioned paper show that the cardiac output of subject *A. G.* at Manitou, Colorado, were essentially the same as those obtained at Baltimore, as given in table 1 of the present paper.

During the course of the present experiments the subject's weight varied only about 3 kgm. As shown in table 1 there was no definite seasonal variation noted in the pulse rate or in the oxygen consumption.

TABLE 1

*The pulse rate, oxygen consumption and cardiac output of A. G., as determined in the recumbent position before arising from bed in the morning*

| DATE | PULSE RATE | OXYGEN CONSUMPTION | CARDIAC OUTPUT | DATE | PULSE RATE | OXYGEN CONSUMPTION | CARDIAC OUTPUT |
|---|---|---|---|---|---|---|---|
| *1928* | *per minute* | *cc. per minute* | *liters per minute* | *1928* | *per minute* | *cc. per minute* | *liters per minute* |
| June 22 | 60 | 230 | 3.83 | December 20 | 64 | 228 | 4.13 |
| June 24 | 62 | 235 | 3.72 | December 23 | 60 | 235 | 4.08 |
| June 26 | 60 | 236 | 3.64 | December 24 | 66 | 232 | 3.78 |
| July 1 | 60 | 228 | 3.95 | December 27 | 58 | 230 | 4.08 |
| July 6 | 62 | 232 | 3.73 | December 29 | 60 | 233 | 3.84 |
| July 8 | 64 | 230 | 4.12 | December 31 | 60 | 230 | 4.14 |
| July 10 | 60 | 234 | 4.08 | *1929* | | | |
| July 12 | 62 | 233 | 4.05 | January 3 | 62 | 235 | 3.95 |
| July 15 | 66 | 230 | 3.92 | January 6 | 62 | 232 | 4.06 |
| July 19 | 60 | 236 | 3.73 | January 10 | 64 | 230 | 3.82 |
| July 23 | 62 | 226 | 3.86 | January 15 | 60 | 228 | 3.95 |
| July 27 | 58 | 228 | 3.98 | January 19 | 56 | 225 | 3.86 |
| August 1 | 60 | 230 | 3.88 | January 23 | 60 | 223 | 3.97 |
| August 7 | 62 | 232 | 3.76 | January 28 | 62 | 226 | 3.90 |
| August 9 | 58 | 233 | 3.81 | February 1 | 64 | 230 | 3.82 |
| August 14 | 64 | 230 | 4.00 | February 5 | 60 | 223 | 3.94 |
| August 18 | 60 | 223 | 3.62 | February 10 | 60 | 224 | 3.90 |
| August 23 | 66 | 225 | 3.75 | February 14 | 66 | 227 | 3.86 |
| August 27 | 60 | 230 | 3.92 | February 17 | 58 | 225 | 3.92 |
| September 4 | 58 | 235 | 4.16 | February 20 | 62 | 230 | 4.00 |
| September 10 | 60 | 230 | 3.85 | February 24 | 66 | 224 | 3.91 |
| September 13 | 60 | 232 | 4.04 | February 28 | 60 | 230 | 3.86 |
| September 16 | 60 | 223 | 4.05 | March 3 | 56 | 232 | 3.75 |
| September 20 | 60 | 226 | 4.13 | March 7 | 64 | 230 | 3.87 |
| October 12 | 62 | 230 | 3.76 | March 12 | 62 | 230 | 3.98 |
| October 15 | 63 | 230 | 3.88 | March 15 | 64 | 234 | 3.96 |
| October 20 | 60 | 228 | 4.09 | March 19 | 60 | 236 | 3.90 |
| October 24 | 58 | 226 | 3.92 | March 22 | 60 | 234 | 4.06 |
| October 29 | 60 | 232 | 3.99 | March 25 | 66 | 226 | 3.87 |
| November 2 | 62 | 236 | 3.73 | March 28 | 64 | 228 | 3.85 |
| November 6 | 64 | 236 | 3.92 | April 8 | 58 | 224 | 3.78 |
| November 10 | 66 | 230 | 3.80 | April 11 | 54 | 226 | 3.63 |
| November 14 | 67 | 233 | 3.83 | April 16 | 60 | 223 | 3.72 |
| November 18 | 60 | 230 | 3.94 | April 20 | 60 | 228 | 3.84 |
| November 21 | 60 | 226 | 3.65 | April 24 | 62 | 232 | 3.93 |
| November 26 | 57 | 225 | 3.60 | April 27 | 56 | 234 | 3.95 |
| November 30 | 60 | 228 | 3.72 | April 30 | 60 | 236 | 3.86 |
| December 2 | 62 | 230 | 3.73 | May 3 | 60 | 235 | 4.07 |
| December 5 | 65 | 230 | 3.78 | May 8 | 60 | 230 | 4.08 |
| December 10 | 60 | 227 | 3.80 | May 12 | 62 | 226 | 3.94 |
| December 15 | 62 | 225 | 3.69 | May 15 | 60 | 224 | 3.85 |

TABLE 1—*Concluded*

| DATE | PULSE RATE | OXYGEN CONSUMPTION | CARDIAC OUTPUT | DATE | PULSE RATE | OXYGEN CONSUMPTION | CARDIAC OUTPUT |
|---|---|---|---|---|---|---|---|
| *1929* | *per minute* | *cc. per minute* | *liters per minute* | *1929* | *per minute* | *cc. per minute* | *liters per minute* |
| May 20 | 58 | 225 | 3.89 | November 9 | 60 | 226 | 3.83 |
| August 31 | 64 | 223 | 3.90 | November 14 | 62 | 230 | 3.95 |
| September 3 | 60 | 232 | 3.96 | November 16 | 64 | 228 | 3.90 |
| September 7 | 62 | 230 | 3.98 | November 21 | 62 | 230 | 3.80 |
| September 13 | 66 | 234 | 4.07 | November 25 | 60 | 226 | 3.72 |
| September 16 | 64 | 230 | 4.13 | December 3 | 58 | 223 | 3.76 |
| September 20 | 60 | 226 | 4.05 | December 7 | 60 | 224 | 3.78 |
| September 24 | 60 | 224 | 3.90 | December 10 | 60 | 226 | 3.87 |
| September 29 | 58 | 227 | 4.12 | December 15 | 60 | 230 | 3.89 |
| October 3 | 64 | 230 | 3.84 | December 18 | 61 | 232 | 3.95 |
| October 7 | 60 | 228 | 3.89 | December 20 | 60 | 230 | 3.90 |
| October 12 | 62 | 226 | 3.73 | December 31 | 60 | 224 | 3.73 |
| October 16 | 60 | 230 | 4.12 | *1930* | | | |
| October 20 | 62 | 224 | 4.02 | January 2 | 60 | 230 | 3.65 |
| October 24 | 64 | 226 | 4.06 | January 4 | 60 | 226 | 3.83 |
| October 30 | 66 | 230 | 3.93 | January 7 | 62 | 234 | 4.10 |
| November 3 | 60 | 228 | 3.74 | January 9 | 60 | 228 | 3.74 |

The results of the cardiac output measurements on *A. G.* given in table 1 require but little comment. They show that when proper precautions are taken to maintain strictly basal conditions, the *cardiac output of man is constant from day to day and is not subject to daily or seasonal variations.* The results are with rare exceptions within the range of 3.9 ± 0.2 liters. This variation is within the unavoidable error of the experimental procedures employed. The variations in cardiac output induced by a less stringent adherence to basal conditions will be discussed in a later section.

*The variation of the cardiac output of a female subject from day to day.* The general constancy of the cardiac output as observed in the case of the male subject, *A. G.*, would not necessarily be applicable to female subjects where one must consider the interference of the menstrual cycle. A series of determinations was, therefore, carried out on a female subject, *L. C. G.*, similar to those recorded in table 1. The results on the female subject showed the usual wide variation in the oxygen consumption from day to day as found by others who have demonstrated the cyclic nature of this variation and its relation to the catamenial cycle. (Collett and Liljestrand, 1924; Benedict and Finn, 1928; Hitchcock and Wardwell, 1928; Griffith *et alii*, 1929.) The cardiac output also showed wider variations in the daily values than was found for the male subject. It is quite possible that the same variation occurs in the cardiac output as has been found

in the basal metabolism. A catamenial fluctuation of this latter function was denied by earlier investigators and it is only in recent, carefully controlled studies that the existence of this fluctuation has been demonstrated. A similar study of the cardiac output on a number of individuals is necessary before one can conclude definitely as to the existence and nature of this variation. Such a study is comtemplated for the future and the data obtained on the one subject studied during the present investigation shall, therefore, be reserved for future presentation. For the present, it must be concluded that the cardiac output is relatively constant, even for the female subject, and that any menstrual variation, if present, is probably not greater than 10 per cent of the average.

*Conditions necessary for attaining truly basal conditions.* The degree of constancy displayed by the results discussed above was obtained under very stringent basal conditions. A question of great practical importance arises as to the variation which occurs when less stringent conditions are observed. As previously shown, the ingestion of food or liquids, psychic disturbances, and a failure to attain a state of complete relaxation from previous physical endeavour will affect the cardiac output and must be avoided in obtaining data referable to the basal condition. The effect of disturbed or insufficient rest during the night preceding the experiment, the effect of the effort involved in coming to the laboratory before the experiment, or the effect of slight indisposition or malaise, have not been previously determined. During the course of a series of determinations made on subject *A. G.*, during the past two years, an answer to these questions has been obtained. In an experiment performed in bed in the usual manner, but in which the subject had retired only two hours previously, the loss of sleep was reflected in a high pulse rate, blood pressure, and in a greatly increased cardiac output (5.00 liters) as compared to the normal values. Subjectively, too, the subject experienced a certain degree of excitation but was not obviously abnormal. Even slight degrees of malaise, as has also been previously shown (Grollman, 1929f) will cause a considerable rise in cardiac output over the normal value. In certain experiments the subject, after a normal night's rest, came to the laboratory where the experiment was performed. The trip to the laboratory required over an hour's ride in a congested street car. The long preliminary preparations, involving several hours, which ensued from the time of the subject's awakening until the performance of the experiment had a definite effect on the cardiac output, which was usually about 0.5 liter higher than it was normally. Similarly, working in the laboratory before the experiment, even after a subsequent rest of half an hour or more until the pulse rate became constant, often, though not always, caused a deviation of the cardiac output from its basal value. On the other hand, when the subject traveled only a short distance before coming to the laboratory or indulged moderately

in work before the experiment, the cardiac output, after the usual resting period, was essentially the same as under strictly basal conditions. We must thus conclude that the previous activity of the subject can affect the cardiac output and that the latter is in this way much more easily made to deviate from the normal than the oxygen consumption.

Since most physiological experiments are conveniently performed in the laboratory rather than under the stringent conditions under which the data recorded in table 1 were obtained, the experiments of table 2 are cited which include all the determinations performed over a period of a year on a male subject, *L. M.*, who lived within a few minutes' walk from

TABLE 2

*The variation of the cardiac output and its related functions of L. M. in the basal, resting condition*

The subject lived within several minutes' walk from the laboratory and the determinations were made in the sitting posture after one-half to an hour's resting period.

| DATE (1929) | PULSE RATE | BLOOD PRESSURE | OXYGEN CONSUMPTION | CARDIAC OUTPUT |
|---|---|---|---|---|
| | *per minute* | *mm. of Hg* | *cc. per minute* | *liters per minute* |
| February 5 | 74 | 98/70 | 210 | 3 40 |
| February 12 | 72 | 95/60 | 214 | 3 60 |
| February 23 | 66 | | 198 | 3 32 |
| February 25 | 66 | | 212 | 3 40 |
| March 6 | 75 | 95/62 | 206 | 3 43 |
| April 19 | 60 | 98/71 | 204 | 3 42 |
| April 22 | 70 | 102/70 | 200 | 3 33 |
| September 4 | 70 | | 203 | 3 20 |
| September 9 | 72 | | 200 | 3 33 |
| December 4 | 66 | 98/70 | 208 | 3 59 |
| December 7 | 64 | 97/65 | 210 | 3 56 |
| December 10 | 70 | 100/70 | 200 | 3 33 |
| December 12 | 65 | 98/73 | 201 | 3 40 |

the laboratory. This subject came to the laboratory soon after awakening, in the fasting condition, indulged in no work before the experiment, and rested until the pulse rate became constant ($\frac{1}{2}$ to 1 hour). Examination of the data of table 2 shows practically the same degree of constancy as those of table 1, obtained under much more stringent conditions. The same conclusion as to the insignificant effect of dressing and coming to the laboratory (when the journey involved only a short distance) was also reached in a previous study (Grollman, 1929e). It has also been repeatedly observed that if the subject takes breakfast at about 7 a.m., and indulges in moderate activity during the morning, the cardiac output, after about an hour's rest, will resume practically its basal value about 1 p.m. The pulse

rate under these conditions is usually elevated above the normal basal value.

Discussion. The present findings indicate a remarkable constancy of the cardiac output and an absence of any seasonal variation as claimed by Lindhard (1913). In the temperate climate of Baltimore, where the present studies were performed, one would scarcely expect such climatic factors as varying intensity of solar radiation (particularly the ultra-violet radiation, which Lindhard considered the cause of the seasonal variation observed by him) to appreciably affect the circulatory system. Seasonal fluctuations in the environmental temperature might well be reflected in the cardiac output, but these, as has already been stated, were avoided during the present investigation. Collet and Liljestrand's (1924) conclusion regarding the large variation of the cardiac output with the catamenial cycle has also not been substantiated. The cardiac output of their subject varied from 2.6 to 5.0 liters per minute and this large variation was ascribed by the authors to the effect of the menstrual cycle. The results on the female subject studied during the present investigation showed a daily variation of only about 10 per cent from the average.

The results of the present study permit one to outline the conditions necessary for obtaining basal values of the cardiac output. Such basal determinations are essential in clinical studies of the cardiac output. To obtain basal values in such cases it is desirable, as can be done with ease in hospitalized subjects, to make the determination in bed as soon after the subject's awakening in the morning as is practicable. Long delay, without breakfast, may lead to psychic unrest which will affect the cardiac output.

As was demonstrated in an earlier paper (Grollman, 1928) it is more difficult to carry out the rebreathing procedure used in determining the cardiac output in the recumbent than in the sitting posture. This is particularly true of untrained subjects. In order to avoid this difficulty it is advisable to do the actual rebreathing of the acetylene mixture while the subject is sitting up in bed. The determination of the basal metabolism and other functions incidentally studied are done, of course, while the subject is recumbent. The subject sits up in bed immediately before beginning to rebreathe the experimental mixture. Since the whole procedure is completed before a single circulation of the blood, the results obtained for the cardiac output refer to the recumbent position and the conditions assumed by the subject just before the rebreathing procedure.

## SUMMARY

The daily variation of the cardiac output was studied over a period of a year and a half. The determinations were made in bed soon after arising in the morning and under these conditions were found to be constant, within the experimental error of the methods employed, showing a total variation

of less than 10 per cent. Seasonal variations were not observed. Factors which affect the cardiac output and the conditions necessary for obtaining truly basal values are outlined.

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# THE EXCRETION OF CALCIUM THROUGH THE INTESTINE

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There is still much confusion in the literature concerning the question of calcium excretion in tetany. Macallum and Voetglin (1909) found the excretion to be increased in tetany. Cook (1910) was unable to confirm this. Salveson (1923) (1924) and his associates injected calcium intravenously into normal and parathyroidectomised animals and determined the calcium of the feces. Excretion through the intestine was much increased in the operated animals. From this they concluded that the threshold for calcium excretion was lowered by parathyroid removal. Greenwald and Gross (1925a) criticized this conclusion on the basis that the tissues had been saturated by the injected calcium, and the increased excretion represented calcium in excess of that which the tissues could retain.

The manner by which the calcium of the blood is raised after injection of an extract of the parathyroids (Collip) is becoming clearer. Greenwald and Gross (1925b) believe that the hormone induces the passage of calcium from the bones into the circulation. Studies of the bone trabeculae after prolonged administration of the hormone by Bauer, Aub, Fuller and Albright (1929) support this view. Greenwald and Gross found that the fecal calcium of animals kept upon a diet containing a known amount of calcium was increased from 90 to 100 per cent after injections of the extract.

This brief review shows how incomplete is our knowledge upon the subject of the calcium disappearance in tetany and of the hypercalcemia in parathyroid overdosage.

It seemed to us, since in any event we would not expect the differences in excretion of normal and of parathyroidectomised animals or in animals treated with the extract would be large, that methods dependent upon the estimation of the calcium of the feces would possess an error that would obscure the true result. The method used by Stewart and Percival (1927) by which a portion of the intestine was washed out until calcium free, appeared more likely to yield accurate results. This method, with some modifications, was used by us throughout.

We also wished to determine the excretion through different parts of the intestinal tract, and this would have been impossible by the other methods. Excretion through the large bowel, loops of small intestine at different

levels and through the entire length of the small intestine was followed respectively in 1, *normal animals;* 2, *normal animals after the injection of a known quantity of calcium chloride;* 3, *parathyroidectomised animals after the injection of calcium;* 4, *animals treated with parathyroid extract and injected with calcium.*

METHODS. In the earlier experiments upon the small bowel a loop about 30 cm. long was tied off in such a way as to leave the blood and nerve supply as little interfered with as possible. Glass cannulae were passed into the lumen through openings made in either end of the loop and tied in position. The segment was thoroughly flushed out until the final washings were calcium free. At the beginning of the work distilled water was used for washing but it was soon found that this apparently caused damage to the intestinal wall. There was marked distention in some cases, loss of tone of the muscular coats and the passage of large quantities of fluid into the lumen. For this reason warm isotonic calcium-free saline was substituted in all subsequent experiments. Care was exercised to keep the surface of the intestines warm and moist with saline during the operative procedures which took usually from 15 to 20 minutes. After the final washing the loop was sealed at either end by placing rubber caps over the ends of the cannulae, and replaced within the abdomen. The abdominal wound was closed and the animal was allowed to recover from the anesthetic.

From 3 to 6 hours later the abdomen was reopened under an anesthetic and the length of bowel flushed out until the washings were again calcium-free. The calcium content of the total washings was then determined. This gave the calcium excretion for the 3 or 6 hour period. The condition of the bowel at the time of the second washing appeared quite normal, the colour and tone were good and there was no more than a small quantity of fluid in the lumen, which it was presumed represented the normal secretion.

In the later experiments instead of a loop being used, the excretion through the entire small intestine was followed over a six hour period. Cannulae were tied into the lumen immediately below the pylorus and just above the ilio-colic sphincter. The bowel was then flushed out with 600 cc. of fluid or until the final 100 cc. contained no trace of calcium. The cannulae were closed by rubber caps and the further procedure was the same as already described. In the case of the large bowel the same general method was followed. The bowel was tied off at its upper limit and as near its lower end as possible.

The determination of the quantity of calcium in the washings was carried out in the following way. The washings were usually cloudy and contained shreds of mucus and epithelial debris. These were removed by centrifuging and the calcium in the clear supernatant fluid was then deter-

mined by the Kramer-Tisdall (1921) method for serum as modified by Collip and Clark (1925). After the first few experiments it was thought advisable to acidulate the fluid before centrifuging against the possibility that calcium in an insoluble form, such as the carbonate, was being thrown down with the other solid material; 5 cc. of concentrated hydrochloric acid therefore were added to each 100 cc. of the washings. The yield of calcium was only slightly increased by this addition.

1. *The excretion of calcium through the large and small bowels respectively.* The excretion of calcium through the large bowel of normal cats kept upon the usual diet of meat scraps and dog biscuit varied from a trace to 0.33 mgm. in periods of from 3 to 5 hours. The excretion through the entire small bowel varied from a trace to 1.2 mgm. When a loop 30 cm. in length,

TABLE 1

*Excretion of calcium through the bowel of normal animals on a diet of meat scraps and dog biscuit*

| ANIMAL | REGION OF INTESTINAL CANAL | NORMAL EXCRETION OF CALCIUM | PERIOD OF TIME OVER WHICH OBSERVATIONS WERE MADE |
|---|---|---|---|
| | | *mgm.* | |
| Cat 4 | Distal 30 cm. of small bowel | 0.16 | 4.5 hours |
| Cat 5 | Distal 30 cm. of small bowel | 0.38 | 3.5 hours |
| Cat 11 | 30 cm. distal to pylorus | 0.61 | 3 hours |
| Cat 13 | Entire small bowel | Trace | 3 hours |
| Cat 75 | Entire small bowel | 1.2 | 6 hours |
| Cat 76 | Entire small bowel | Trace | 6 hours |
| Cat 6 | Large bowel | 0.15 | 5 hours |
| Cat 7 | Large bowel | 0.33 | 4 hours |
| Cat 21 | Large bowel | 0.33 | 3 hours |
| Cat 19 | Large bowel | Trace | 3 hours |

or about one quarter the length of the small intestine was used, the excretion amounted to 0.16 in one case, to 0.38 and 0.61 respectively in two others. When such very small amounts of calcium are involved the error is comparatively large, and only an approximation can be hoped for. The results of this series are shown in table 1.

2. *The excretion of calcium after the intravenous injection of calcium chloride.* The procedures already described were carried out in this series, but after the first or preliminary washing of the bowel 60 mgm. per kilo of calcium chloride (about 22 mgm. of Ca) were injected into the femoral vein. Five minutes later a sample of blood was removed from the carotid, or as in later experiments, from the femoral artery and the calcium content of the serum determined. After a period of three hours in the earlier experiments and of six hours in the later ones the excretion through different

parts of the intestinal tract was estimated. The blood calcium was again determined at this time.

The serum calcium was found to have been raised over 100 per cent by the injection. The injected calcium, however, disappeared from the blood in a remarkably short time. In three or in six hours, according to the length of the after period, the serum calcium had again dropped to the normal level of from 10 to 12 mgm. (tables 2 and 3). One would naturally

TABLE 2

*Excretion through the large intestine after the intravenous injection of calcium chloride into normal animals*

| ANIMAL | WEIGHT | $CaCl_2$ INJECTED | SERUM CALCIUM | | CALCIUM EXCRETED | |
|---|---|---|---|---|---|---|
| | | | 5 minutes after injection | 6 hours after injection | Calcium in first washing at beginning of experiment | Calcium in second washing 6 hours later |
| | *grams* | *mgm.* | *mgm. per 100 cc.* | *mgm. per 100 cc.* | *mgm.* | *mgm.* |
| Cat 37 | 2,950 | 180 | 26 | 11.9 | 0 | 1.2 |
| Cat 39 | 2,500 | 150 | 23.4 | 9.5 | 0 | 1.6 |
| Cat 40 | 4,100 | 250 | 19.2 | 11.8 | 0 | 0 |

TABLE 3

*Excretion through the small intestine after injection of calcium into normal animals*

| ANIMAL | WEIGHT | $CaCl_2$ INJECTED | SERUM CALCIUM | | CALCIUM EXCRETED | |
|---|---|---|---|---|---|---|
| | | | 5 minutes after injection | 6 hours after injection | Calcium in first washing at beginning of experiment | Calcium in second washing 6 hours later |
| | *grams* | *mgm.* | *mgm. per 100 cc.* | *mgm. per 100 cc.* | *mgm.* | *mgm.* |
| Cat 48 | 2,150 | 125 | 21.5 | 10.4 | 0 | 0.54 |
| Cat 51 | 2,500 | 150 | 22.5 | 10.5 | 0 | 0.30 |
| Cat 64 | 1,800 | 115 | 24.8 | 12.9 | 0 | No Ca |
| Cat 65 | 2,550 | 165 | 25.4 | 10.4 | 0 | Trace |
| Cat 70 | 3,150 | 210 | 30.1 | 12.2 | 0 | 1.0 |
| Dog 1 | 2,000 | 120 | 19.1 | 12.4 | 0 | 0.77 |

suppose that the disappearance of the injected calcium was accomplished by excretion through the intestinal mucosa and the kidneys. Greenwald and Gross (1925b) found an increased excretion associated with the hypercalcemia which follows the administration of repeated doses of parathyroid extract, while Stewart and Percival (1927) reported a loss of a large part of the injected calcium through the mucous membrane of the large bowel; the latter observers concluded that the main excretory route for the injected calcium was through this portion of the intestine. The figures which they publish, however, scarcely bear out this conclusion. For instance, 500 mgm.

of calcium chloride were injected intravenously, which raised the calcium of the blood to 21 mgm. per 100 cc. of serum. In 3 hours the serum calcium had dropped to 10 mgm. and the excretion of calcium into the large bowel during this time amounted to 14 mgm. Over 150 mgm. of injected Ca remained unaccounted for with the exception of a small amount excreted in the urine. No observations upon the excretion through the small bowel were reported.

Our results do not agree with the findings of these investigators. The excretion of calcium through either the large or the small bowel was not significantly greater, except in one case for which we believe there is an explanation, than in the previous series on normal animals which had received no injections. The disappearance of the injected calcium from the blood in such a short space of time could not be accounted for by its excretion through either the bowels or the kidneys. In the one case in which there was an apparent increase in the excretion through the bowel, this had quite evidently sustained damage. Distilled water had been used for washing and the permeability of the intestinal wall, we believe, had been affected. The bowel was markedly distended and filled with a large quantity of fluid which we think was more of the nature of a transudate than a true secretion. As already mentioned, distilled water seemed always to have a more or less injurious effect upon the bowel wall and was soon discarded as a wash fluid. In no case in which the bowel was of good tone and colour, and never when saline was used for washing, was there a significant increase in the calcium excretion after its injection into the veins.

The comparatively rapid disappearance of the excess calcium from the blood is an observation which awaits an explanation. It does not leave the body, so far as we have been able to determine. In some animals the kidneys were tied off after the injection, but this apparently had no effect upon the disappearance of the calcium from the blood or upon the excretion through the intestine. In another experiment the liver was sequestered from the circulation by tying off the hepatic artery and the portal vein, and the removal of the intestinal tract. The fall in the blood calcium was just as abrupt and profound as when the liver remained intact. Though the organ is not of course completely isolated from the circulation by this operation its functions are very greatly depressed, as evidenced by the fall of the blood sugar to the convulsive level.

For the most part, the determinations of the calcium excretion without the injection of calcium and the determinations following the injection of calcium were compared in different animals. But in some cases both determinations were made upon the same animal. The normal excretion was first determined for a 3 hour period, calcium was then injected and a second determination was made after a second 3 hour period. The results

corroborated those obtained in the previous series. The excretion during the second period was practically the same as during the first; this is shown in table 4.

3. *The excretion of calcium in parathyroidectomized animals after the intravenous injection of calcium chloride.* The greater number of the experiments in this series were carried out upon cats. Thyroparathyroidectomy was performed under ether anesthesia. Twenty-four hours later the excretion of calcium through the intestinal tract was determined after the injection of calcium into the femoral vein. The excretion of the injected calcium through the intestinal wall was increased in all cases after removal of the parathyroids. Three experiments were carried out upon the large bowel. The increase in the excretion over the normal was of a moderate degree through this portion of the intestine. But the excretion through the small bowel, and it was upon this region of the intestinal tract that the

TABLE 4

*Excretion of calcium through the large and the small bowels for three-hour periods before and after the injection of calcium intravenously*

| ANIMAL | WEIGHT | EXCRETION IN FIRST 3-HOUR PERIOD | $CaCl_2$ INJECTED | SERUM CALCIUM | | CALCIUM EXCRETION AFTER SECOND 3-HOUR PERIOD | REGION OF BOWEL |
|---|---|---|---|---|---|---|---|
| | | | | 5 minutes after injection | 3 hours after injection | | |
| | *grams* | | *mgm.* | *mgm. per 100 cc.* | *mgm. per 100 cc.* | | |
| Cat 13 | 3,300 | 0.23 | 200 | 20 | 11.6 | 0.20 | Large intestine |
| Cat 21 | 3,600 | 0.33 | 220 | 22 | 13.0 | 0.10 | Large intestine |
| Cat 19 | 3,500 | Trace | 220 | 28.4 | 11.8 | 0.10 | Large intestine |
| Cat 23 | | Trace | 150 | 20.4 | 9.4 | Trace | Small intestine |

greater number of observations were made, was very markedly increased. Over a period of 6 hours it amounted to from 4 to 10 mgm. The excretion was also determined upon two pups of the same litter. The normal excretion after calcium injection was determined upon one and found to be around the usual figure, 0.77 mgm. in 6 hours. The other was parathyroidectomised. Twenty-four hours after the operation the serum calcium had fallen and the excretion was 4.43 mgm. The results of this series are shown in table 5.

It will be noticed that the serum calcium level is not greatly lowered by parathyroidectomy. In our experience the calcium is not depressed to the same degree in cats after removal of the glands as it is in dogs. The majority of the animals showed a drop of from 10 to 20 per cent in the calcium level, and very few showed definite tetanic symptoms. In some 100 cats upon which we have performed parathyroidectomy in the past two years weakness, loss of appetite and lassitude were the most notice-

able effect, and these are no doubt due in part at least to the thyroid deprivation.

4. *The effect of repeated doses of parathyroid extract upon the excretion of calcium.* The effect of the extract upon the excretion of calcium has been investigated, so far, on only a small number of animals. "Parathormone"[1] as prepared by Lilly & Co. according to the method of Collip (1925) was used. The animals were given 3 or 4 0.5 cc. doses per day for 3 successive days. We have found as a result of previous work with this preparation that the

TABLE 5

*Excretion of calcium through the small bowel after the injection of calcium into the veins of parathyroidectomized animals*

| ANIMAL | WEIGHT | SERUM CALCIUM | | CALCIUM INJECTED | SERUM CALCIUM | | CALCIUM EXCRETED | |
|---|---|---|---|---|---|---|---|---|
| | | Before parathyroidectomy | 24 hours after parathyroidectomy | | After 5 minutes | After 6 hours | Ca in first washing | Ca in second washing 6 hours later |
| | *grams* | *mgm. per 100 cc.* | *mgm. per 100 cc.* | *mgm.* | *mgm. per 100 cc.* | *mgm. per 100 cc.* | *mgm.* | *mgm.* |
| Cat 49 | 2,000 | 10.0 | 9.7 | 120 | 18.3 | 11.1 | 0 | 4.0 |
| Cat 50 | 2,700 | 9.71 | 9.40 | 160 | 22.4 | 10.4 | 0 | 10.6 |
| Cat 53 | 2,500 | 10.1 | 9.66 | 150 | 20.6 | 10.7 | 0 | 9.0 |
| Cat 63 | 2,600 | 10.2 | 8.5 | 155 | 28.8 | 12.9 | 0 | 5.0 |
| Cat 60 | 3,000 | 10.7 | 9.8 | 180 | 26.4 | 13.1 | 0 | 5.0 |
| Cat 56 | 3,000 | 11.2 | 9.2 | 180 | 23.5 | 10.1 | 0 | 3.4 |
| Cat 61 | 3,300 | 8.8 | 8.2 | 200 | 25.9 | 15.0 | 0 | 9.1 |
| Cat 83 | 1,900 | 10.3 | 9.7 | 115 | 26.6 | 16.3 | 0 | 2.9 |
| Cat 85 | 1,800 | 10.7 | 9.4 | 110 | | 11.84 | 0 | 3.4 |
| Cat 86 | 2,200 | 10.1 | 8.8 | 135 | 33.5 | 11.84 | 0 | 3.0 |
| Cat 87 | 4,100 | 10.5 | 9.1 | 300 | 26.1 | 10.27 | 0 | 4.35 |
| Cat 88 | 3,500 | 10 | 9.6 | 200 | 19.5 | 10.5 | 0 | 1.5 |
| Cat 92 | 2,700 | 10 | 9.3 | 160 | | 11.6 | 0 | 1.9 |
| Dog 1 | 2,000 | 12.1 | | 120 | 19.1 | 12.4 | 0 | 0.77 |
| Dog 2 | | 13.6 | 9.4 | 105 | 17.8 | 14.2 | 0 | 4.43 |

maximum effect upon the blood calcium was reached after this period and with this frequency of dosage. In the animals of these experiments the symptoms were well marked at this time and the blood calcium had reached the level of about 18 mgm. per 100 cc. of serum. In a series of 5 animals investigated the excretion of calcium through the bowel was not increased above the normal by the extract. If anything it was slightly less than the normal. Greenwald and Gross (1925b), it may be recalled, obtained a greater excretion of calcium in the feces of dogs treated with "Parathormone." Our results, however, agree with those of Stewart and Percival

[1] We have much pleasure in thanking the Eli Lilly Co. for supplying us with this material.

who also obtained a slight fall in the excretion. In our cases the divergence from the normal is too small and the animals employed too few in number to enable us to arrive at a definite conclusion with regard to any depressing effect of the hormone upon excretion. That it did not increase the loss of calcium by the bowel seems however quite definite. It may be mentioned that Hunter and Aub (1927) found increased excretion of calcium in the urine and to a less extent in the feces of human cases of lead poisoning after treatment with the hormone. Albright, Bauer, Rapes and Aub (1929) observed an increased urinary excretion when "parathormone" was administered over long periods to subjects on a calcium deficient diet. The fecal excretion was not increased.

Discussion. The results of our experiments with the intravenous injection of calcium chloride into normal animals show that the calcium disappears rapidly from the blood and apparently is stored up in some part of the body. Its disappearance cannot be accounted for even in part by excretion through the bowels or kidneys, and evidently it is not removed from the circulation by the liver. It disappears just as quickly from the blood of the eviscerated animal. Some tissue, it would seem, is possessed of an unusual avidity for calcium. Bone and muscle at once suggest themselves as the tissues that are probably responsible for the removal of the element from the blood. Direct proof of this, as by calcium determinations of the tissues concerned, is difficult to obtain on account of the small quantities which disappear as compared with the large amounts of calcium which must be present in the tissues of the entire animal, especially is this so in the case of bone. It would be interesting to determine how far the storing up process can be carried. A time must come when the tissues shall have become saturated, and it is likely that excretion will then ensue, or symptoms develop as a result of the overloading of the tissues.

No attempt has been made to determine the slope of the calcium curve or to ascertain the precise time that this takes to reach the normal level. It cannot, however, be far short of 3 hours, for in most cases the calcium was still slightly in excess at this time. There is evidently a very rapid disappearance of the injected calcium immediately after injection, for analyses made 5 minutes after this never gave as high determinations as one would expect considering the comparatively large quantities employed. In cat 21 for instance, (table 4) which weighed 3600 grams the blood volume, taking the rather high figure of 7 per cent of the body weight as a basis, would amount to about 250 cc. or roughly 125 cc. of serum. Five cubic centimeters of fluid containing 220 mgm. of calcium chloride or about 88 mgm. of Ca were injected. Since 100 cc. of serum contain between 10 and 11 mgm. of Ca before injection we would expect that the 88 mgm. added would raise the serum calcium to the level of over 70 mgm. per 100 cc. The figure actually obtained in this and other cases is less than

one-third of this. It would appear therefore that there is an almost immediate migration of calcium from the blood into the tissues.

Our results with parathyroidectomised animals are in agreement with the conclusions of Salveson. The difference in the amounts excreted in normal and in the operated animals was very definite. The fall in the blood calcium following parathyroidectomy as a rule was not very marked, but in cases where the blood calcium did not drop at all, as when we presume the parathyroid tissue was not completely removed, there was no change in the excretion. On the other hand, parathyroid removal resulting in a lowering of blood calcium was always accompanied by increased excretion. Control operations in which the muscles of the neck were handled and the parathyroids exposed but not removed, had no effect upon the excretion. Greenwald (1911) found a distinct phosphorus retention in tetany, and has sought to show that Salveson's results are secondary to high blood phosphate. He found the excretion of calcium in the feces to be greater when it is injected in combination with sodium phosphate than when given alone. A series of two experiments upon one animal only were reported. It may be mentioned that in a series of experiments carried out by us in the manner described in this paper and to be reported later, high blood phosphorus was found to have an insignificant effect upon calcium excretion.

## SUMMARY

1. There is a small but constant excretion of calcium through the small and the large bowels of normal cats.

2. Calcium injected into the veins of animals kept upon the usual diet of meat scraps and biscuit practically disappears from the blood within 3 hours.

3. The disappearance cannot be accounted for by excretion through the bowels or kidneys. So far as we have been able to determine, the excess calcium does not leave the body.

4. The excretion of the injected calcium through the large and the small bowels is several times greater in parathyroidectomised than in normal animals.

5. The administration of parathyroid extract was found to be without significant effect upon the excretion of calcium through the bowel during a 6 hour period. This was slightly less than that found in normal animals, but the difference was slight and cannot be considered significant.

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# BLOOD FIBRIN STUDIES

## The Concentration of Fibrin Yielded by Canine Plasma in Relation to Dietary Factors[1]

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The present investigation of the relation of diet to the production of fibrinogen has been attempted under dietary conditions which were intended, as far as possible, to permit the more precise definition as well as modification of the food intake. Nearly all of the results obtained in previous studies have been complicated by the inadvertent introduction of several variables owing to the complex character of the rations used.[2]

Methods. Quantitative methods for the determination of fibrin have been reviewed by several workers, Gram (1921), Foster and Whipple (1922a), Howe (1925), and Starlinger (1923). The method of Foster and Whipple is very satisfactory when only fibrin is to be determined, and was used here with minor modifications: 1. The blood, drawn under aseptic conditions, was delivered into 1 cc. of a 1.6 per cent sodium oxalate solution. A 1.6 per cent solution is more nearly isotonic with dog's blood and contains a sufficient excess of oxalate to prevent the sample from coagulating should more than the usual 9 cc. be drawn at one time. 2. All samples were centrifuged for 45 minutes at 3000 r.p.m. 3. The saline coagulating mixture contained 0.8 per cent NaCl and 0.125 per cent $CaCl_2 \cdot 2H_2O$, and was used in the proportions of 45 cc. of "saline" to 2 cc. of oxalated plasma.

The formulae of Gram (1921) were employed in all calculations.

In all the feeding experiments the blood samples were drawn from the

[1] The data in this paper are taken from a dissertation presented by Harry M. Vars in partial fulfillment of the requirements for the degree of Doctor of Philosophy, Yale University, 1929.

Part of the expenses were defrayed by a grant from the Russel H. Chittenden Fund for Research in Physiological Chemistry.

[2] Lack of space prevents the presentation of any historical review. The references are scattered, but the following authors have discussed various phases of the problem: Gulliver (1846), Lehmann (1851), Nasse (1842a), (1842b), Poggiale (1847), Mathews (1899), Chajes (1904), Böhme (1911), Reiss (1913), Adler (1918), Hanson and McQuarrie (1917–18), Utheim (1920), Epstein (1917), Foster and Whipple (1922b, c), Howe (1925), Schultz, Nicholes and Schaefer (1925), Mark (1926).

jugular vein. As a check upon the accuracy of the method blood was drawn from both jugular veins at approximately the same time, and treated under the standard conditions adopted. Variations of 5 to 6 per cent in the figures for duplicate analyses is considered as the limit of accuracy of the method, though in the majority of instances the values checked closer than this.

There is conflicting evidence[3] as to the relative concentration of fibrinogen in the blood from different vessels. Mathews (1899) observed that the mesenteric vein is constantly richer in fibrinogen than arterial blood and carotid blood contained more than the blood from the vena cava, both

TABLE 1

*Comparison of the fibrin content of heart (arterial) and jugular (venous) blood by the methods of Foster and Whipple (1922a) and Schultz et al. (1925); dog 8, casein III diet*

| DATE | WEIGHT | FOSTER-WHIPPLE METHOD | | | | SCHULTZ ET AL. METHOD | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | Arterial blood | | Venous blood | | Arterial blood | | Venous blood | |
| | | Fibrin per 100 cc. | Cells | Fibrin per 100 cc. | Cells | Fibrin per 100 cc. | Cells | Fibrin per 100 cc. | Cells |
| | *kilos* | *mgm.* | *per cent* | *mgm.* | *per cent* | *mgm.* | *per cent* | *mgm.* | *per cent* |
| December 21 | 16.32 | 199 | 51.0 | 200 | 52.3 | 243 | 50.7 | 229 | 51.5 |
| December 23 | 16.03 | 188 | 55.1 | 198 | 56.2 | 209 | 54.3 | 211 | 54.3 |
| December 27 | 15.80 | 215 | 53.1 | 204 | 54.0 | 239 | 53.6 | 232 | 53.5 |
| December 30 | 15.85 | 197 | 51.3 | 196 | 51.8 | 226 | 50.5 | 221 | 50.3 |
| January 2 | 16.20 | 157 | 51.0 | 162 | 51.6 | 178 | 50.0 | 184 | 51.4 |
| January 5 | 16.10 | 182 | 55.0 | 180 | 54.4 | 202 | 53.6 | 207 | 54.4 |
| Average | | 190 | | 190 | | 216 | | 214 | |

Note: About 20 cc. of blood were drawn, in one syringe, from each vessel and then placed in two tubes for analysis by both methods. Jugular blood was always drawn first.

above and below the kidney. Jugular blood is said to yield roughly 7 to 15 per cent less fibrin than blood from the left ventricle (Schultz et al., 1925). The method employed by Schultz and his collaborators gives slightly higher values than those obtainable by the Foster-Whipple procedure. Table 1 presents a study of the relative concentration of fibrin yielded by jugular and heart blood, by the above two methods. There is a very close parallelism in the fibrin values for both vessels, with higher concentrations being recorded where the method of Schultz et al. is employed.

[3] For earlier references see Lehmann (1851), Brown-Séquard (1858), Dastre (1893). This earlier data, however, must not be interpreted as conclusive in view of the methods of experimentation available.

To study more completely the possible variation in fibrin concentration from vessel to vessel 7 animals were used. The jugular vein and the vessels in the abdomen were exposed, under amytal or ether anesthesia, after which the various samples were drawn and subjected to analysis. Only the results of one experiment will be recorded, table 2, as they all show the same general picture. On the basis of the data collected, the conclusion is justified that the differences, where present, are mostly within the limits of experimental error.

*Experimental animals and the diets used.* Dogs, usually females, weighing 6 to 26 kilos, in good nutritive condition and free from infection,

TABLE 2

*Fibrin content of blood from different vessels. Dog 1, 202. kilos. Anesthetic—65 mgm. amytal per kilo*

| TIME | VESSEL | CELLS | FIBRIN AVERAGE | FIBRIN PER 100 CC. | |
|---|---|---|---|---|---|
| | | | | Plasma | Blood |
| | | *per cent* | *mgm.* | *mgm.* | *mgm.* |
| 10:55 | Jugular vein | 37.2 | 6.8 | 396 | 257 |
| 11:23 | Vena cava | 34.6 | 6.9 | 401 | 262 |
| 11:28 | Mesenteric vein | 36.9 | 6.7 | 393 | 248 |
| 11:30 | Portal vein | 36.4 | 6.6 | 386 | 246 |
| 11:32 | Hepatic vein | 37.4 | 6.8 | 404 | 253 |
| 11:34 | Jugular vein | 34.6 | 6.9 | 402 | 262 |
| 11:35 | Heart | 36.3 | 6.6 | 385 | 245 |
| 11:39 | Vena cava | 36.4 | 6.7 | 383 | 243 |
| 11:40 | Mesenteric vein | 36.6 | 6.7 | 392 | 249 |
| 11:41 | Portal vein | 41.4* | 6.7 | 397 | 233 |
| 11:41:30 | Hepatic vein | 37.2 | 7.2 | 419 | 272 |
| 11:42 | Jugular vein | 38.0 | 6.7 | 394 | 244 |
| 11:42:30 | Heart | 41.0* | 6.6 | 390 | 230 |
| | Heart | 36.9 | 7.1 | 419 | 264 |

* Because of the much higher cell volume, these two samples must have clotted slightly before the blood and oxalate were adequately mixed. The fibrin values bear this out. If, however, we assume that the average cell volume is 37 per cent, we get on calculation 245 mgm. and 242 mgm. of fibrin, respectively, in whole blood—figures agreeing closely with the other samples.

were kept in individual metabolism cages in accordance with the general plan described by Cowgill (1923). No dietary observations are reported here from animals that were known to be infected, in as much as infections have long been known to cause marked changes in the fibrin content of the blood.

The animals were bled three times a week. The loss of blood (approximately 30 cc.) is not sufficient to cause a disturbance in the fibrin level as a result of hemorrhage for Foster and Whipple (1922b) have shown that a

37 pound dog could be bled 40 cc. daily for about five weeks with no untoward effect.

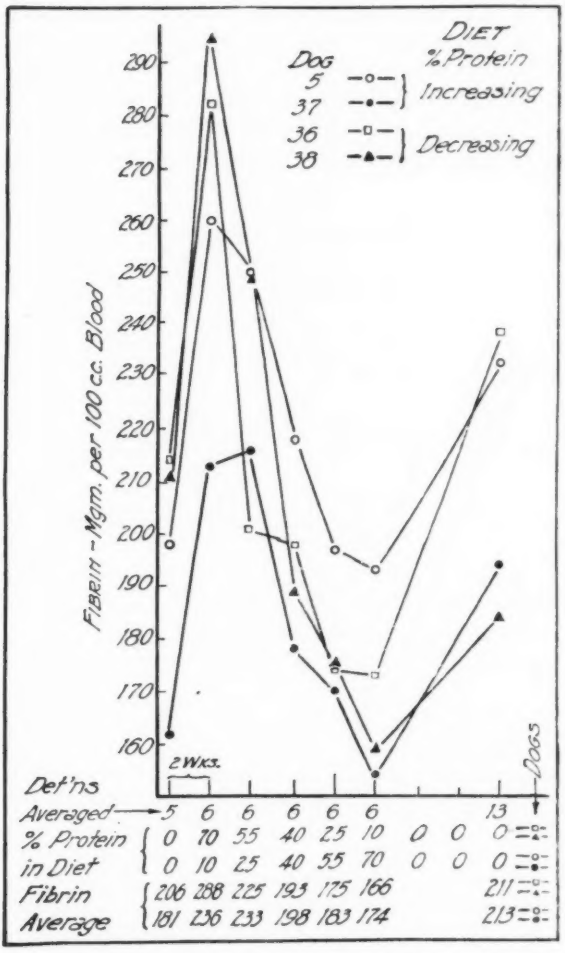


Chart 1. Average fibrin values for 4 dogs fed different amounts of protein (casein) for periods of 2 weeks, and then fed a "no protein" diet for 6 weeks. Note the similarity in the shapes of the curves irrespective of whether the percentage of protein in the diet was increasing or decreasing.

As a rule the animals were offered "artificial" food mixtures, adequate in every known respect, and in amounts greater than they would eat. In

some experiments the food pans were left in the cages all of the time except for the period new food was being weighed, about one hour, while in others the food was available to the animal eight hours daily. When animal tissues were fed, they were supplemented by salts, bone ash, and accessory food substances as supplied by a wheat germ product,[4] in the proportions that the animals received these materials in the "synthetic" rations. Cod liver oil was used as the source of vitamins A and D only in the low fat diet (see table 7), and when animal tissues were offered. To insure the complete consumption of the supplements they were fed with a small por-

TABLE 3

*Composition of typical diets*

| | CAS. III | "NO PROT." V5 | "NO PROT." V6 | "NO PROT." V7 | CAS. 10B | CAS. 25B | CAS. 40B | CAS. 55B | CAS. 70B |
|---|---|---|---|---|---|---|---|---|---|
| | *per cent* | *per cent* | *per cent* | *per cent* | *per cent* | *per cent* | *per cent* | *per cent* | *per cent* |
| Casein* | 41.18 | | | | 10.00 | 25.00 | 40.00 | 55.00¶ | 70.00¶ |
| Meat residue† | | | | | | | | | |
| Sucrose | 29.41 | 32.60 | 33.65 | 33.33 | 44.45 | 36.00 | 30.40 | 15.40 | 0.39 |
| Dextrin | | 11.90 | 12.05 | 19.40 | 16.00 | 9.40 | | | |
| Starch | | | | | | | | | |
| Lard | 18.30 | 17.80 | 18.35 | | | | | | |
| Butter | 7.19 | 7.70 | 7.95 | 26.30 | 25.65 | 25.65 | 25.65 | 25.65 | 25.65 |
| Bone ash | 2.61 | 4.75 | 4.89 | 6.06 | 2.64 | 2.64 | 2.64 | 2.64 | 2.64 |
| Salt mixture‡ | 1.30 | 4.45 | 1.51 | 1.51 | 1.32 | 1.32 | 1.32 | 1.32 | 1.32 |
| Cellu flour§ | | 20.80 | 21.40 | 13.40 | | | | | |

* A commercial product containing 13 per cent N.

† A commercial product obtained from the Valentine Meat Juice Co., Richmond, Va.

‡ Salt mixture no. 2. See G. R. Cowgill, 1923, This Journal, lxvi, 164.

§ A wood fiber flour obtained from the Chicago Dietetic Supply House, Chicago, Ill.

¶ To obtain a ration of the proper consistency the casein was moistened with warm water, then dried, and ground to a very coarse powder. The other dry ingredients were added and the whole was held together by the melted fat.

tion of the main food. After this was ingested the remaining food was offered. Water was freely available.

The vitamins B and G were supplied by "Vitavose"[4] which was fed in quantities of 0.6 to 1.0 gram per kilo of body weight, an amount sufficient to satisfy the dog's requirement (Cowgill, Deuel, and Smith, 1925).

All of the "synthetic" rations were prepared on the "kilo unit" basis after the directions of Cowgill (1923). Table 3 gives the composition of

[4] "Vitavose," a commercial product obtained from E. R. Squibb and Sons, New York.

typical diets. It will be seen that the protein content of the "B" series of rations varied widely, all of this variation being accomplished by shifting the amount of carbohydrate, the sum percentage of protein and carbohydrate remaining the same. The same plan was followed in making up the "meat residue"[5] diets. The numerical part of the name designating each protein-containing diet, e.g., casein 40B refers to the per cent of protein in the ration; the letter B following such a number indicates that all of the fat supplied was in the form of butter.

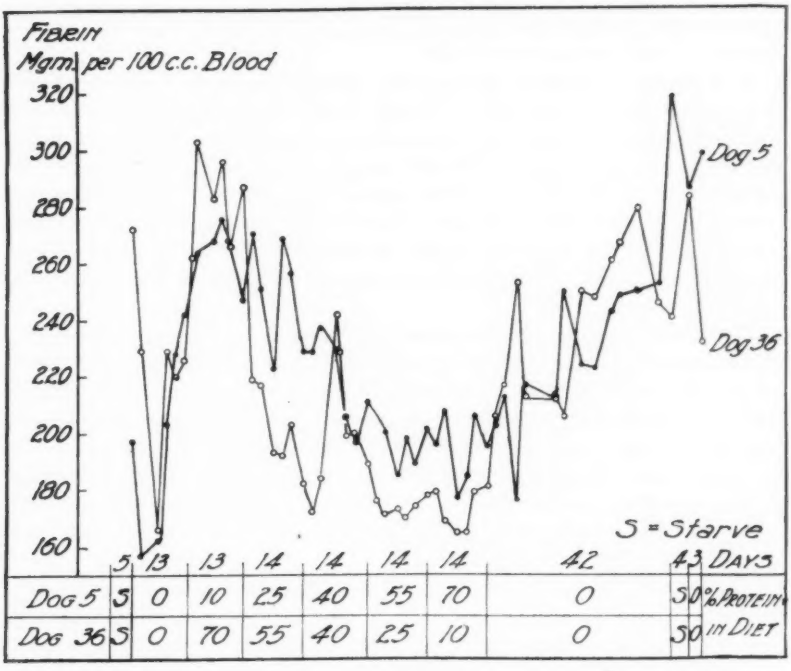


Chart 2. Typical fibrin curves for 2 dogs upon dietary régimes in which only the protein content of the diet was varied. See chart 1 for the *average* fibrin values of these two animals during the same period.

In all experiments a record was kept of the daily food consumption, which, in connection with the weight record, gave a good index of the nutritive condition of the animals.

*Effect of dietary factors upon the fibrinogen concentration of canine*

[5] A commercial product obtained from the Valentine Meat Juice Co., Richmond, Va.

*plasma*. The protein portion of the ration is the most logical dietary component for immediate consideration. There are few economically available proteins suitable for feeding larger animals over extended periods, and of these the best are commercial casein and "meat residue." These materials are relatively constant in composition, and when fed at the proper level, are adequate with respect to the known essential amino acids. Both protein foods were used with practically the same success. Only in the case of some of the diets rich in "meat residue," e.g., Meat Res. 70B, was there any indication that the animals were not eating as much as one would expect. However, the results in both groups of animals were consistent and checked each other.

It is essential that the experimental periods should be long enough to give an accurate picture of the effects of the ration under consideration. It soon became apparent that a short observational period, say 3 to 4 days, is apt to give an entirely different impression from that obtained over longer periods. From chart 2 it is readily apparent that there can be wide variations in the results obtained, if analyses are not continued for some time. The feeding of animals over a period of ten days to two weeks may, however, be expected to afford fairly definite conclusions as to the effect of the diet in question.

It is of interest to study the effect of a "no protein"[6] diet upon the blood fibrin picture, for such a basal ration gives a means of studying the effect of a single variable, the protein. Theoretically there is much to recommend a protein-free regimen, for we can be sure that the animal has been deprived of only one nutrient, protein, while with complete inanition, on the other hand, the picture is complicated by having a multiple deficiency of all essentials as well as protein starvation, so that the response observed when the first food is ingested may be due to any one of the several materials present in the ration.

Foster and Whipple (1922b) state that "Fasting conditions give more stability to the blood fibrin values and the physiological variations are less. The average fibrin values are less in fasting than during periods of mixed diet." Table 4 shows that, in general, fasting does lower the average fibrin value for each dog, but that the level attained is not always the same, this depending perhaps upon the ration employed previous to the period of inanition. Because of such variations and the fact that complete starvation does not give an ideal base line for the study of the protein alone of the diet, the practice of starving the animals between dietary studies was abandoned, in favor of the "no protein" rations.

The one feeding experiment to be described was planned to answer the

[6] Diets as reported in table 2 are as low in N as it is practicable to use in the type of experimentation described here, and hence, for convenience, are designated as "no protein" diets.

TABLE 4

*Effect of complete inanition on the fibrinogen content of blood*

| DOG | PERIOD OF STARVATION | DIET PRECEDING STARVATION | PERIOD ON DIET | FIBRIN PER 100 CC. BLOOD | | |
|---|---|---|---|---|---|---|
| | | | | Before starvation | After starvation | Change |
| | *days* | | *days* | *mgm.* | *mgm.* | *mgm.* |
| 2 | 4 | Dog biscuit | 60+ | 212 | 185 | −27 |
| 2 | 4 | Casein 75 | 49 | 193 | 168 | −25 |
| 2 | 5 | Casein III | 35 | 218 | 210 | −8 |
| 2 | 6 | Pig stomach | 15 | 209 | 186 | −23 |
| 2 | 6 | No protein V6 | 40 | 315 | 317 | +2 |
| 1 | 3 | Casein III | 45 | 320 | 290 | −30 |
| 4 | 4 | Dog biscuit | 30+ | 150 | 160 | +10 |
| 4 | 4 | Casein 75 | 42 | 204 | 185 | −19 |
| 4 | 5 | Casein III | 35 | 184 | 140 | −40 |
| 4 | 4 | Pig kidney | 9 | 226 | 232 | +6 |
| 4 | 6 | No protein V6 | 48 | 275 | 269 | −6 |
| 3 | 4 | Dog biscuit | 30+ | 175 | 192 | +17 |
| 3 | 4 | Casein 75 | 42 | 159 | 141 | −15 |
| 3 | 5 | Casein III | 30 | 235 | 180 | −55 |
| 3 | 6 | Pig liver | 15 | 370 | 225 | −145 |
| 3 | 6 | No protein V6 | 40 | 270 | 296 | +22 |
| 7 | 4 | Dog biscuit | 30+ | 174 | 181 | +7 |
| 7 | 5 | Pig stomach | 14 | 210 | 161 | −49 |
| 7 | 5 | No protein V6 | 49 | 301 | 308 | +7 |
| 6 | 5 | Casein III | 31 | 215 | 176 | −39 |
| 6 | 6 | Pig liver | 15 | 205 | 179 | −26 |
| 6 | 6 | No protein V6 | 40 | 266 | 276 | +10 |
| 5 | 5 | Casein III | 35 | 195 | 145 | −50 |
| 5 | 4 | Pig kidney | 9 | 201 | 165 | −36 |
| 5 | 6 | No protein V6 | 47 | 281 | 286 | +5 |
| 27 | 4 | Dog biscuit | 30+ | 465 | 379 | −86 |
| 36 | 4 | No protein V7 | 43 | 241 | 284 | +43 |
| 37 | 4 | No protein V7 | 43 | 307 | 362 | +55 |
| 5 | 4 | No protein V7 | 43 | 319 | 287 | −32 |
| 38 | 4 | No protein V7 | 43 | 220 | 387 | +167 |
| 39 | 5 | Meat residue 13 | 41 | 345 | 322 | −23 |
| 40 | 5 | Casein 40B | 34 | 177 | 192 | +15 |
| 41 | 5 | Casein 40B | 28 | 164 | 179 | +15 |
| 42 | 5 | Casein 40B | 28 | 148 | 170 | −22 |
| 23 | 3 | Dog biscuit | 14 | 344 | 324 | −20 |
| | 5 | Dog biscuit | 14 | 324 | 383 | +60 |

following questions. What would animals do if, after a short period on a "no protein" diet, they were placed for equal periods of time on rations containing increasing percentages of protein,[7] i.e., 10, 25, 40, 55, 70 per

[7] In the diets the sum percentage of protein plus carbohydrate was always the same, 70.4 per cent, hence any increase in protein was accompanied by a corresponding decrease in carbohydrate.

cent, all other essential ingredients of the diet being the same? Would animals show the same response after a similar pre-period on a "no protein" diet if the order of feeding the protein diets was reversed, i.e., 70, 55 per cent, etc.? It is conceivable that with the former plan of feeding there would be a step-like increase in fibrin with each increment of protein added to the ration, and in the latter there would be decreasing values after the first sharp rise. Both proteins, casein and "meat residue" were fed to eight dogs, two animals being placed on each dietary régime.

Each food mixture was fed for a period of two weeks, as previous experience showed that such a period may be expected to give fairly accurate conclusions as to the effect of the ration on blood fibrin. Typical data are summarized in chart 1, which gives the results with the casein diets only. The animals on the "meat residue" rations presented a very similar picture. Chart 2 presents the fibrin curves for two representative

TABLE 5

*Effect of continued "no protein" dietaries on blood fibrin*

| DOG | TOTAL DAYS ON DIET | "NO PROTEIN" V6 DIET | | | | DOG | TOTAL DAYS ON DIET | "NO PROTEIN" V7 DIET | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Fibrin averages (per 100 cc. blood) | | | | | | Fibrin averages (per 100 cc. blood) | | | |
| | | For days | Fibrin (determinations) | For days | Fibrin (determinations) | | | For days | Fibrin (determinations) | For days | Fibrin (determinations) |
| | | | *mgm.* | | *mgm.* | | | | *mgm.* | | *mgm.* |
| 3 | 39 | 1–18 | 198(9) | 18–39 | 232(9) | 36 | 41 | 1–16 | 213(6) | 16–41 | 254(6) |
| 4 | 46 | 1–18 | 177(9) | 18–46 | 284(11) | 37 | 41 | 1–20 | 148(6) | 20–41 | 244(6) |
| 5 | 47 | 1–13 | 185(6) | 13–47 | 282(15) | 5 | 41 | 1–14 | 204(5) | 14–41 | 251(8) |
| 6 | 37 | 1–16 | 149(8) | 16–37 | 239(10) | 38 | 41 | 1–20 | 169(7) | 20–41 | 203(6) |

animals on the casein diets, and indicates the type of data that have been averaged to produce the composite curves of chart 1.

The inclusion of protein in the diet has undoubtedly stimulated the production of the increased amount of fibrin. All animals show an appreciable rise in fibrin values after the preliminary period on the "no protein" ration, amounting to an average increase of 55 mgm. per cent in the animals receiving 10 per cent protein as contrasted to an 82 mgm. per cent increase in animals receiving 70 per cent protein. It was surprising that the casein 70B diet did not produce a larger increment than it did, only about 35 per cent more than did the casein 10B ration. During the next four periods, or eight weeks, the fibrin concentration dropped more rapidly in those animals receiving decreasing amounts of protein.

Rations containing a high percentage of protein when fed over long periods do not cause the fibrin values to remain at a high level. This suggests that the ingested protein may act as some sort of transient stimu-

TABLE 6

*Effect of animal tissue diets on the fibrinogen content of blood*

| DATE | WEIGHT | CELLS | FIBRIN PER 100 CC. BLOOD | DATE | WEIGHT | CELLS | FIBRIN PER 100 CC. BLOOD |
|---|---|---|---|---|---|---|---|
| Pig stomach | | | | | | | |
| | *kgm.* | *per cent* | *mgm.* | | *kgm.* | *per cent* | *mgm.* |
| 1/23 | 6.08 | 43.7 | 218* | 1/ 5 | 14.32 | 49.7 | 174† |
| 28 | 5.60 | 43.9 | 210** | 9 | 13.80 | 50.5 | 207†† |
| 30 | 6.01 | 44.0 | 266 | 10 | 14.00 | 50.5 | 205 |
| 2/ 2 | 6.25 | 41.5 | 251 | 11 | 13.90 | 49.2 | 201 |
| 3 | 6.43 | 52.5 | 236 | 12 | 14.20 | 50.5 | 205 |
| 6 | 6.47 | 44.4 | 215 | 13 | 14.18 | 49.8 | 200 |
| 8 | 6.90 | 42.1 | 235 | 14 | 14.42 | 48.4 | 208 |
| 10 | 6.92 | 39.8 | 224 | 16 | 14.39 | 49.0 | 188††† |
| 12 | 7.03 | 45.3 | 209 | 17 | 14.96 | 47.9 | 218 |
| 18 | 6.00 | 49.0 | 186*** | 18 | 15.08 | 45.3 | 245 |
| | | | | 19 | 15.25 | 48.6 | 224 |
| | | | | 20 | 15.55 | 50.0 | 182 |
| | | | | 21 | 15.44 | 48.2 | 222 |
| | | | | 22 | 15.30 | 49.5 | 221 |
| | | | | 23 | 15.56 | 47.9 | 210 |
| | | | | 28 | 14.30 | 54.9 | 161†††† |
| Pig liver | | | | | | | |
| 1/23 | 7.13 | 45.3 | 215§ | 1/23 | 8.69 | 40.2 | 235 △ |
| 28 | 6.30 | 52.9 | 176§§ | 28 | 7.80 | 44.8 | 180 △△ |
| 30 | 6.78 | 56.4 | 212 | 30 | 8.13 | 43.7 | 194 |
| 2/ 1 | 6.85 | 52.6 | 200 | 2/ 1 | 8.45 | 38.8 | 209 |
| 3 | 7.16 | 53.1 | 203 | 3 | 8.75 | 37.9 | 251 |
| 6 | 7.14 | 53.1 | 215 | 6 | 8.81 | 46.9 | 334 |
| 7 | 7.07 | 49.9 | 248 | 7 | 9.10 | 39.6 | 336 |
| 9 | 7.33 | 49.7 | 238 | 9 | 8.98 | 41.0 | 303 |
| 11 | 7.40 | 48.6 | 233 | 11 | 8.88 | 40.0 | 374 |
| 12 | 7.31 | 53.8 | 205 | 12 | 8.88 | 40.9 | 370 |
| 18 | 6.52 | 53.7 | 179§§§ | 18 | 7.99 | 46.5 | 225 △△△ |

*Pig stomach diets:*

* Dog 2. After 35 days on casein III diet.
** Starved 1/23–1/28. Started pig stomach diet (ad lib.).
*** Starved 2/12–2/18.
† Dog 7. After 4 months on dog biscuit diet.
†† Starved 1/5–1/9. Started pig stomach diet (500 gm.).
††† Increased pig stomach diet to 1000 gm.
†††† Starved 1/23–1/28.

*Pig liver diets:*

§ Dog 6. After 31 days on casein III diet.
§§ Starved 1/23–1/28. Started pig liver diet.
§§§ Starved 2/12–2/18.
△ Dog 3. After 31 days on casein III diet.
△△ Starved 1/23–1/28. Started pig liver diet.
△△△ Starved 2/12–2/18.

TABLE 6—*Concluded*

| DATE | WEIGHT | CELLS | FIBRIN PER 100 CC. BLOOD | DATE | WEIGHT | CELLS | FIBRIN PER 100 CC. BLOOD |
|---|---|---|---|---|---|---|---|
| Pig kidney | | | | | | | |
| | *kgm.* | *per cent* | *mgm.* | | *kgm.* | *per cent* | *mgm.* |
| 1/23 | 11.96 | 49.5 | 195‡ | 1/23 | 8.00 | 46.2 | 184¶ |
| 28 | 11.00 | 50.5 | 145‡‡ | 28 | 7.25 | 49.4 | 140¶¶ |
| 30 | 11.62 | 50.3 | 236 | 30 | 7.46 | 49.5 | 211 |
| 2/ 1 | 11.61 | 47.5 | 224 | 2/ 1 | 7.69 | 47.5 | 243 |
| 3 | 11.82 | 43.6 | 238 | 3 | 7.92 | 45.5 | 265 |
| 6 | 11.88 | 51.5 | 201 | 6 | 8.12 | 49.7 | 226 |
| 10 | 11.00 | 49.7 | 165‡‡‡ | 10 | 8.00 | 48.0 | 234¶¶¶ |

*Pig kidney diets:*

‡ Dog 5. After 35 days on casein 75 diet.
‡‡ Starved 1/23–1/28. Started pig kidney diet.
‡‡‡ Starved 2/6–2/10.
¶ Dog 4. After 35 days on casein III diet.
¶¶ Starved 1/23–1/28. Started pig kidney diet.
¶¶¶ Starved 2/6–2/10.

lus to increased fibrinogen production. Apparently the type of protein fed does not play any part in determining the response elicited; the same general results were obtained with the widely unlike casein and "meat residue."

Of equal interest is the rise in blood fibrin when the animals are continued on a "no protein" diet for 5 to 7 weeks. In general, animals show a gradual decrease in blood fibrin concentration during the first two weeks on such a diet, after which there is an increase. Table 5 presents the data of two experiments. The results for the "no protein" V7 ration as shown graphically in chart 1 do not show this initial decline and then the final rise in values though these are apparent in table 5. The nature of the reaction causing this rise is not known.

The duration of the initial decline makes the use of the two-weeks' feeding period of more significance as a proper preliminary treatment before studying the effect of the inclusion of protein in the diet.

Further evidence as to whether the nature of the protein fed plays a definite rôle in determining the fibrin values is presented in table 6, which tabulates the results obtained when animal tissues were ingested. Foster and Whipple (1922b) noted that animal-protein diets favored high fibrin levels, and that cooked pig stomach caused a profound increase, up to 100 per cent, above the fasting level. The animals, however, were fed the animal-proteins only 2 to 4 days, after a pre-period of starvation.

In these experiments fresh tissues, pig's stomach, kidney, and liver, were washed, boiled one hour in water, drained, and put through a grinder.

Boiling caused about a 25 per cent loss in weight. Not more than a three days' supply was ground at any one time, and was kept in the ice-box until used. Generally the dogs were offered amounts in excess of that they would eat, unless otherwise noted in the table. The method of feeding the dietary supplements has been described previously.

TABLE 7

*Average fibrin values for the different experimental diets*

| DIET | NUMBER OF DOGS USED | TOTAL DAYS ON DIET | TOTAL DETERMINATIONS | GRAND AVERAGE | LOW AVERAGE* (DETERMINATIONS) | HIGH AVERAGE* (DETERMINATIONS) |
|---|---|---|---|---|---|---|
| | | | | *mgm. per cent* | *mgm. per cent* | *mgm. per cent* |
| Low fat | 1 | 23 | 8 | 232 | | |
| Casein 10B | 4 | 56 | 24 | 201 | 159(6) | 260(6) |
| Casein 25B | 4 | 56 | 24 | 204 | 174(6) | 250(6) |
| Casein 40B | 7 | 83 | 51 | 193 | 178(6) | 218(6) |
| Casein III | 16 | 245 | 82 | 230 | 136(3) | 284(9) |
| Casein 55B | 4 | 56 | 24 | 203 | 170(6) | 249(6) |
| Casein 70B | 4 | 54 | 24 | 232 | 154(6) | 294(6) |
| Casein 75 | 5 | 182 | 53 | 204 | 174(12) | 234(8) |
| Meat residue 13 | 3 | 89 | 32 | 283 | 259(10) | 297(10) |
| Meat residue 25 | 2 | 90 | 31 | 264 | 217(17) | 320(14) |
| Meat residue 10B | 3 | 82 | 27 | 244 | 184(7) | 274(13) |
| Meat residue 25B | 3 | 46 | 18 | 257 | 222(6) | 286(6) |
| Meat residue 40B | 3 | 42 | 18 | 252 | 198(6) | 331(6) |
| Meat residue 55B | 3 | 44 | 18 | 245 | 200(6) | 321(6) |
| Meat residue 70B | 3 | 71 | 18 | 250 | 195(6) | 353(6) |
| No protein V5 | 4 | 95 | 30 | 175 | 148(7) | 202(9) |
| No protein V6 | 6 | 236 | 118 | 229 | 199(18) | 246(18) |
| No protein V7 | 7 | 257 | 95 | 223 | 162(6) | 341(9) |
| Pig kidney | 2 | 18 | 10 | 212 | 207(5) | 217(5) |
| Pig liver | 2 | 30 | 18 | 249 | 214(9) | 283(9) |
| Pig stomach | 2 | 29 | 23 | 205 | 208(14) | 231(8) |
| Dog biscuit | 9 | 300+ | 26 | 188 | 136(3) | 248(2) |

* These columns report the range of "individual variation" in the experimental subjects. The data for each animal were averaged separately, and the figures show the lowest and highest averages recorded for individuals on the diet in question. The number in parenthesis indicates the total determinations on the animal.

The two animals receiving pig stomach for 14 and 15 days respectively, certainly did not show any variation in fibrin values outside that offered on preceding rations of casein. With liver as a source of protein, dog 3 showed a marked rise in fibrin, while dog 6 exhibited what might be termed a normal reaction. There was no evidence that infection or other extraneous factors was causing the exceptional rise in dog 3. During the nine day period of feeding pig kidney to dogs 4 and 5, there was a gradual rise and then a decline in fibrin concentration.

These data warrant the conclusion that pig stomach, under the experimental conditions imposed here, may be no more effective in causing changes in the fibrinogen content of the blood than is pig liver or kidney.

*Average fibrin values.* Table 7 summarizes all of the data collected from animals on different dietary régimes. The striking closeness of all the averages can only be considered as fortuitous. It is apparent from chart 2 that large variations may occur, yet when the average is determined the peaks and depressions are so smoothed out that the final results may be very similar. No extended attempt has been made to treat the figures statistically as there are too many variables, unknown at present, operating to make the conclusions reliable. All of this emphasizes the statement of Howe (1925): "To pick out definite values as representative of particular states, and especially of the normal composition of the blood plasma, is exceedingly uncertain."

## SUMMARY AND CONCLUSIONS

When diets of known composition, in which all the components but the carbohydrate and protein protions remain constant (these two being substituted isocalorically, one for the other), are fed to healthy, vigorous dogs, the concentration of fibrin yielded by the blood will depend upon the nature of the previous regimen and the duration of its feeding. The feeding of different protein-containing diets causes variations in the fibrinogen concentration of the blood. Rations containing a high percentage of protein, when fed over long periods, do not cause the fibrin values to remain at a high level. This seems to indicate that the ingested protein acts as some sort of transient stimulus to increase fibrinogen production. When rations containing gradually increased amounts of protein are fed the concentration of fibrin yielded by the blood does not increase with each increment of protein. Apparently, the type of protein ingested does not play any marked part in determining the response elicited; similar results were obtained with the widely unlike casein and "meat residue." Rations extremely low in protein when fed over long periods also seem to stimulate fibrinogen production.

Under the experimental conditions adopted pig stomach appeared no more effective in causing changes in the fibrin values than did pig liver or kidney. In fact, the variations were no greater than those observed when casein and "meat residue" were fed.

Observational periods of ten days or longer are necessary to secure a clear cut demonstration of the effects of the special diet. Longer periods may profitably be employed, whereas short periods are likely to give an entirely erroneous picture.

The daily variation in fibrin concentration, observed in some periods were less than 10 per cent, though usually they amounted to more than this (20–25 per cent). The reasons for the fluctuations are still unexplained.

Starvation periods of 3 to 6 days may cause minimum fibrin values. The results, however, will depend largely upon the dietary history (type of diet, and duration of its feeding) immediately preceding the period of inanition.

In a study of the possible variation in fibrin concentration of blood from vessel to vessel, no difference was noted between arterial (heart) and venous (jugular) blood and the variations that were noted between other vessels were mostly within the limits of experimental error.

I wish to express my thanks to Prof. Lafayette B. Mendel for suggesting the general problem and furnishing invaluable advice and suggestions throughout the investigation.

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# STUDIES IN GLANDULAR METABOLISM

## II. The Carbohydrates of Resting and Secreting Submaxillary Glands

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Studies on the metabolism of glands have yielded no definite information regarding the mechanism supplying the energy required for secretion. That carbohydrate may be involved has already been suggested by Anrep and Cannan (1922) who demonstrated a consistent removal of blood sugar by the submaxillary gland at rest. In activity they observed the glucose consumption to be increased approximately in proportion to the rate of salivary secretion. As the saliva contains little or no glucose, it must have disappeared either by direct oxidation or by the anaerobic process of glycolysis. There are some indications that the energy for secretion may arise anaerobically. Bottazzi (1920) demonstrated that the salivary gland of the octopus is capable of secretion, even when oxidations are prevented by NaCN, and showed that after exhaustion the ability to secrete is restored on washing with sea water. Barcroft and Piper (1912) found that the oxygen consumption of the submaxillary gland is greatly increased, not only during secretion, but also for some minutes following activity. Furthermore the intake of oxygen reaches a maximum after the flow of saliva has ceased, as occurs during recovery following the anaerobic phase of muscular contraction. In the previous paper of this series (Himwich and Adams, 1929) it was demonstrated that in glands as in muscle the lactic acid produced on incubation is derived from glycogen. Since it is possible that the processes of the gland during life are different from those of surviving tissue, an attempt was next made to determine whether glycolysis affords energy for glandular secretion.

Method. Four series of experiments were performed to investigate the anaerobic cleavage of glycogen as a possible source of energy for the secreting submaxillary gland. In the first and second series, the carbohydrate balance of the glands was studied. The blood supply to the gland was left intact in the first series, while in the second group of experiments the glands were rendered anaerobic by severing all the blood vessels of the gland. The third and fourth series consisted of studies made of the blood and saliva of the resting and stimulated glands.

Dogs under amytal anesthesia were used and secretion was produced by stimulation of the chorda tympani nerve with a tetanizing current. The saliva was collected by means of a cannula placed in Wharton's duct, and when the flow of saliva had entirely ceased the gland was removed. When the glands were without blood supply, stimulation was continued for ten minutes, although after a few drops of saliva in the first moments no further secretion appeared. In the first and second series, both stimulated and resting glands were weighed immediately upon removal, minced in iced 95 per cent alcohol, and then analyzed for glycogen, intermediary carbohydrates, and lactic acid as previously described (Himwich and Adams, 1929).

After the saliva was collected and its total volume measured, 5 cc. portions were placed in 50 cc. of 95 per cent alcohol and allowed to stand over night. On the following day that part precipitated by alcohol was separated from the extract by filtration. The solid portion was treated as were the glands in analyzing for glycogen, while the alcoholic extract was analyzed for lactic acid and soluble carbohydrates as described for tissues. In the present paper only the results of the analysis of the saliva for lactic acid are considered, since the others are not germane to the argument.

The third and fourth series of experiments were performed to determine the effect of glandular secretion on the lactic acid contents of the blood and saliva. In order to obtain only submaxillary blood, each vein coming from the gland (2 to 5 in number) was carefully exposed, and all the blood vessels emptying cephalad to the orifices of these veins were tied off. The blood from the submaxillary gland could thus be drawn from the external jugular vein and was collected in a Luer syringe while the rate of flow was timed with the aid of a stop watch. Samples of arterial blood were collected simultaneously from the femoral artery. Both venous and arterial blood samples were placed in oxalated flasks to prevent clotting. After removal of the proteins with Somogyi's (1929) reagents, glucose was determind by the Hagedorn-Jensen (1923) method. Following the precipitation of glucose and other interfering substances by $CuSO_4$ and $Ca(OH)_2$, as recommended by Salkowski (1879) and Van Slyke (1917), lactic acid was determined by the procedure of Friedemann, Cotonio, and Shaffer (1923). In the third series the saliva was treated in precisely the same way as the blood in analyzing for lactic acid. In the last eight experiments of this series the hemoglobin contents of the samples of arterial and venous blood were determined by the use of a Haden hemoglobinometer equipped with a glass standard. Dr. Anna J. Eisenman has informed us in a personal communication that the values obtained by this apparatus are directly proportional to those secured by direct determination of oxygen capacity, the latter being 120 per cent of those obtained by the Haden hemoglobinmeter. Since we were interested only in the relative concentrations of two samples, this method was entirely satisfactory.

The lymph flow of the salivary gland was calculated according to the method of Barcroft and Kato (1922) as follows: With the aid of a stop watch the venous blood flow was determined during collection in a Luer syringe. The rate of flow of saliva was also obtained, the saliva being allowed to drop into a flask from a cannula inserted in Wharton's duct. The flow of saliva per minute was added to that calculated for the venous blood in one minute, giving a figure representative of the arterial blood flow if the loss of fluid for lymph was disregarded. This will be called $A_1$. $A_2$, or the actual arterial blood flow, was calculated by multiplying the rate at which the venous blood flowed per minute by the ratio of the hemoglobin concentration of the venous blood to that of the arterial concentration. Thus, $A_2$ included the additional fluid lost to lymph as well as that to saliva, while $A_1$ took into consideration only that lost in saliva. The difference between $A_1$ and $A_2$ was taken as the lymph flow per minute.

The fourth series differed from the third in so far as the stimulation was intermittent, continuing for three seconds every $\frac{1}{4}$ minute and the blood samples were drawn 1 hour, $1\frac{1}{2}$ hours, and 2 hours after stimulation commenced.

RESULTS AND DISCUSSION. In the present study the formation of additional lactic acid was considered the chief criterion for the occurrence of the process of glycolysis. It was assumed that if lactic acid was produced it could be detected in one of the following tissues: *a*, accumulated in the gland; *b*, in the venous blood leaving the gland; *c*, in the saliva; or *d*, in the lymph.

If lactic acid was found in none of the tissues mentioned, the possibility of its oxidation or reconversion to glycogen as rapidly as formed, might still remain.

*a. Lactic acid in the gland.* Previous observations (Himwich and Adams, 1929) have demonstrated that the lactic acid contents of each of a pair of submaxillary glands are approximately the same. Glycogen also appears in practically equal amounts in pairs of glands. The present study of the lactic acid, glycogen, and intermediary carbohydrate contents of the submaxillary gland before and after electrical stimulation revealed no consistent changes. If the energy for secretion is provided by glycolysis, an increase of lactic acid and a decrease of glycogen should take place in the glandular contents during secretion. However, in only two of seven pairs did the stimulated gland contain definitely more lactic acid than the gland at rest. In the other five experiments the differences in lactic acid content of two glands of a pair were negligible, varying between 1 mgm. and 7 mgm. per 100 grams of tissue. If lactic acid was being formed it was apparently being removed too rapidly for detection. In one case, a fall of glycogen of 281 mgm. per 100 grams of tissue was observed, but since the amount of glycogen was considerably higher in this experiment than in any

other, this difference cannot be considered as generally representative. Decreases in glycogen varying between 16 and 58 mgm. per cent were detected in five other experiments. In the remaining pair the glycogen contents of each of a pair of glands were within the experimental error of 25 mgm. per cent.

In order to eliminate the possibility of escape of lactic acid into the blood stream, four additional experiments were performed in which the blood supply was entirely cut off. Lactic acid was significantly increased in only one instance, and glycogen was observed to decrease twice. Two other observations showed slight increases of lactic acid in the stimulated gland. These differences, however, were less than 25 mgm. per cent, and were considered to be within the error of the method. The experiments of this last group are open to the criticism that the glands may have been injured by lack of blood supply following the severing of the blood vessels. Though there were indications from analyses of the glands that lactic acid might be increased during secretion, no conclusive proof of this was obtained.

*b. The lactic acid content of the blood.* Analysis of the blood leaving the gland furnished no evidence of an increased production of lactic acid during activity. In considering the lactic acid contents of arterial and venous blood, a difference of 5 mgm. per 100 cc. of blood was regarded as significant. In the majority of cases the hemoglobin concentrations were determined and the observed lactic acid contents of the arterial blood were corrected by multiplication by the ratio of the hemoglobin concentration of the venous blood to that of the arterial blood. This was necessary since the venous blood was more concentrated due to a loss of fluid in the lymph and saliva.

Of seven observations made during the first minute of secretion, only one showed the gland to be producing lactic acid. This result was not corrected for the change in concentration. In four cases there was no change in lactic acid content, while the other two stimulated glands appeared to remove lactic acid from the blood. One of these was also uncorrected. Twenty-two comparisons were made of blood samples drawn during more prolonged activity, the range being from the second to the twelfth minute of stimulation. From these evidence for the formation of lactic acid during secretion was furnished in only two instances. By far the largest number, sixteen, demonstrated no change in the lactic acid of the blood after its passage through the stimulated submaxillary gland. In the four remaining cases the venous blood contained even less lactic acid than the arterial blood. No evidence for the liberation of lactic acid from glands was obtained in 6 other observations in which blood was drawn 5 and 11 minutes after secretion ceased.

At Professor Anrep's suggestion additional experiments were performed

in order to determine the effect of prolonged intermittent stimulation of the gland. However, these observations in which stimulation was continued for two hours also disclosed no increase of lactic acid in the venous blood of the gland. The fact that no lactic acid was liberated by glands is all the more striking since many of the observations revealed a removal of glucose from the blood in confirmation of the work of Anrep and Cannan (1922).

*c. The lactic acid content of saliva.* Examinations of saliva revealed amounts of lactic acid lower than those found in blood. Two different methods of purification were used; lower results being obtained by one method than by the other. Of seven experiments in which Meyerhof's (1921) method of purification as recommended for tissues was used, amounts of lactic acid were observed ranging from 5 to 27 mgm. per 100 cc. of saliva with an average of 12 mgm. per cent. When interfering substances were removed by Somogyi's (1929) protein precipitation followed by the removal of glucose with Salkowski's (1879) and Van Slyke's (1917) reagents, the average amount found in seven experiments was 3 mgm. per 100 cc. of saliva. It seems conceivable that the benzene extractions of Meyerhof's (1921) procedure failed to remove other bisulphite combining substances which might have been determined as lactic acid by the method of Friedemann, Cotonio, and Shaffer (1927). By either method saliva apparently contained less lactic acid than blood, which was found from 72 observations made in the third series of these experiments, to vary between 9 and 45 mgm. per cent with an average of 21 mgm. per cent. In all experiments where the blood and saliva were both analyzed, the lactic acid found in the saliva was less than that of the blood of the same animal. There is, therefore, no evidence in this part of the system for an increase of lactic acid during glandular secretion.

*d. Lactic acid in the lymph.* The lactic acid present in the lymph was not determined but by estimating the lymph flow as described previously under "method" it was found that very little lactic acid could have left the gland through that channel. From twenty-four such computations, twelve revealed no lymph flow whatever. The values obtained from the other twelve varied from 0.06 to 5.8 cc. of lymph per minute with an average of 1.17 cc. per minute. With such a slight flow, no large amounts of lactic acid could have been present in the lymph. Barcroft and Kato (1915–16) found little or no flow during rest, while during activity in four experiments the flow was 1.23 cc. per minute in the fifth minute, and in another case 0.8 cc. in the 6th minute. Observations taken at later periods disclosed much slower flows.

*e. Immediate oxidation.* If glycolysis is the process by means of which glands derive energy for the work of secretion, lactic acid must be formed in large quantities. Since no significant amounts of lactic acid were found accumulated in the gland, in the blood stream, in the lymph or saliva,

the only conceivable ways in which it could have disappeared were immediate oxidation or reconversion to glycogen. This is not in agreement with Barcroft and Piper's (1912) observation that the maximum oxygen consumptions occurred some minutes after activity. If an anaerobic process is involved in secretion, it does not seem to be that of glycolysis.

These observations of submaxillary gland *in situ* are in sharp contradistinction to previous ones in which glycolysis was found to be a characteristic of surviving submaxillary tissue.

## CONCLUSIONS

Since it was impossible to demonstrate an increased production of lactic acid during secretion of the submaxillary gland, we are forced to the conclusion that glycolysis does not serve as a source of glandular energy. It is to be noted, however, that if the lactic acid produced by glycolysis was entirely reconverted to glycogen or oxidized as soon as it was formed, it could not be detected by our methods.

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# THE REABSORPTIVE FUNCTION OF THE TUBULE IN THE FROG'S KIDNEY[1]

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Studies on tubular reabsorption in the kidney carried out by means of the injection of various substances into the tubules via the ureter have not yielded conclusive evidence. Most of the early work is reviewed by Cushny (1926). The work of Lepine and his students (1886–1912) along these lines has, however, been more or less overlooked. They attempted to injure the tubular epithelium of one kidney in rabbits by injecting quinine salts 4:1000 and mercuric chloride 1:4000, using the opposite kidney as a control. They found that the kidney with injured tubules excreted a greater volume of urine, a decreased amount of urea and an increased amount of chloride and sugar. The studies of Weld (1919), Hinman and Lee Brown (1924), and others, on "Pyelovenous Back Flow," however, might tend to invalidate their findings. Pyelovenous back flow, on the other hand, has not been observed in the frog's kidney (observations from this laboratory, and those of Gile (1927)). Further evidence for tubular reabsorption in the frog's kidney has been reported by Wearn and Richards (1922–24), and by White and Schmidt (1926).

The studies reported in this paper were carried out on the kidneys of large, living frogs, Rana catesbiana. The frogs were anesthetized and prepared for study as previously described (1924, 1929), and cannulae were inserted into the ureters.

By means of blue dye solutions and india ink, the effect of pressure and duration of the injected solution was first studied. One can inject the ureters and larger collecting ducts at a pressure of 5 to 10 cm. of water for 1 to 5 minutes, many of the distal convolutions at 15 to 20 cm. water, practically all of the distal convolutions at 20 and 25 cm. of water for one minute, and proximal convolutions and glomeruli, in addition, at higher pressures.

[1] The experiments herein reported form part of the thesis, "Contributions to the Physiology of the Kidney" submitted in May, 1929, to the Graduate School of the University of Minnesota, by the author, in partial fuifillment of the requirements for the degree of Doctor of Philosophy.

The question of absorption of dyes by the tubular epithelium was next studied. In a series of six frogs each for phenolsulphonephthalein 0.4 per cent and sodium indigosulphonate one per cent solution, the dye solution was injected into the left ureter at a pressure of 20 to 25 cm. of water for one minute. By observing the living kidney during and immediately after the injection, it could be seen that the distal convolutions were almost all or slightly more than half injected with the colored solution and somewhat distended. The proximal convolutions also showed the dye in an occasional loop, so that it can be concluded that the segment between these loops was also somewhat injected. In the case of frogs receiving phenolsulphonephthalein via the left ureter, the urines being excreted by the right kidneys showed this dye in from 4 to 8 minutes or on an average of 5.7 minutes. During the next 15 minutes the concentration of the dye increased so that it was a deep pink upon alkalinization. On the other hand, the urines from the right kidneys receiving sodium indigosulfonate via the left ureter, contained only a very faint trace of this dye which could first be detected 15 to 25 minutes (average 19 minutes) after the injection. These urines presented only a faint greenish blue coloration when held over a white background. In several more frogs in which both of these dyes were studied alternately, the same findings were obtained. In several more series of frogs, indigo-carmine and trypan blue, each a 1 per cent solution, were studied. In both cases the findings paralleled the results obtained with indigosulfonate, namely, it required from 15 to 20 minutes for a just visible trace of the dye to appear in the opposite urines which did not increase in concentration appreciably during thirty or more minutes. These findings would indicate 1, that phenol red is quickly transported from the tubular lumen to the blood stream which then carries it to the opposite side where it is quickly excreted; and 2,that sodium indigosulfonate, indigocarmine and trypan blue are very slowly transported from the tubular lumen to the blood stream and then excreted by the opposite kidney only in small amounts.

Because of the possibility of the epithelium of the ureter and collecting ducts being responsible for what appears to be reabsorption of phenol red, this dye solution was injected into the ureter and collecting ducts of another series of frogs, as above, at pressures of from 5 to 10 cm. of water for from one to five minutes. In no case did the opposite urine show more than a just visible trace of the dye upon alkalinization, and this only after a period of from 15 to 35 minutes.

Thus it can be seen that this excretion of phenolsulphonephthalein by the opposite kidney was due primarily to a reabsorption of the dye from structures more proximal than the ureter and larger collecting ducts. Just which segment is primarily responsible cannot be answered at this time. White and Schmidt (1926) have shown that the proximal convolution in

the Necturus kidney reabsorbs NaCl and sugar. Edwards and Marshall (1924) have shown that the cells of the proximal convolution in the frog's kidney contain large amounts of phenolsulphonephthalein during the excretion of this dye by the kidney. This might therefore indicate that these cells are the ones primarily responsible for this reabsorption.

Studies of the concentration of blue dye solutions injected into the proximal and distal convolutions of the tubules were now made. The dye solutions used were sodium indigosulfonate, indigo-carmine and trypan blue, from 0.25 per cent to 0.05 per cent. The injections were made, as a rule, immediately after the surgical preparation of the frog was completed. At this time, as has been shown by Bieter (1929), the glomerular blood flow is at a minimum, probably due to a reflex inhibition. At a pressure of 30 cm. of $H_2O$ for one minute, the proximal convolutions, visible on the dorsal surface of the kidney, are fairly well injected. During an interval of 30 minutes, some deepening of the color of the dye solutions in the tubular lumina can be observed provided the contents are not washed on by the formation of glomerular filtrates. The amount of concentration, however, as measured by the increased depth of color, is not very great. This lack of marked concentration of the contents of the proximal convolutions might mean that the dye is being reabsorbed at this point.

On the other hand, if the dye solutions are injected into the ureter at the same pressure or at pressures of 20 to 25 cm. $H_2O$ for one minute, and the ventral surface of the kidney is observed with a microscope, a different picture can be seen. The distal convolutions seem to show a greater percentage of injections and this holds especially in the lower half of the kidney. If now the depth of colors in the lumina are observed closely, it can be seen that within 5 minutes many tubules show colors up to about twice as deep as many of the others, and as the process continues, this deepening of color increases to the point when after one-half to one hour, the lumina can be observed as very fine streaks of deep color. Some tubules appear to show relatively little change during one-half to one hour, but the majority, in the kidney of a frog in good condition, show some degree of concentration.

In many tubular lumina of the distal convolutions there appears, as the above concentration proceeds, a precipitation of the dye. In some cases, the granules are very small and seem to be deposited on the inner borders of the cells. In other cases, these masses appear more or less as casts distributed along a portion of the tubule. Finally, all that appears to remain in the lumina are these dye masses, large or small. Occasionally, also, cells can be seen in the lumina stained with the dye. In no case has any staining of the tubular epithelium been observed. This precipitation of the dye particles, which has not been observed to occur in all of the tubules of a given kidney, may be due to the actions of various salts, or it may be due

to the removal of water from the solutions; undoubtedly both factors play a rôle, but it does seem to be associated with the fact that before it occurs, some deepening of the color in the lumina is to be observed. In a series of 8 frogs it has been found that the precipitation of dye and cast formation began to occur from 5 to 20 minutes after the injection—for solutions of the above dyes from 0.25 to 0.05 per cent, respectively. Following the first appearance of these masses, the number occurring in the various lumina increased so that in 20 to 40 minutes almost all of the lumina showed these findings.

While observing the epithelium of the distal convolutions, another interesting finding was made. If the thickness of the epithelium is noticed immediately after the dye injection is made, it can be seen to be very thin. As the above concentration progresses, the epithelium increases in diameter. The diameter of the lumina also gradually decreases so that from this shrinking alone the cells should show an increased diameter. But at a time when the lumina shows a diameter half of what it was immediately after the injection was made, the tubular epithelium appears to be as large, and even larger, than when the lumina is completely obliterated and the tubule is in a more or less resting stage. A number of measurements of the diameters of the epithelium of the distal convolution have been made with a calibrated eyepiece. A single set of tubules has been observed throughout for these readings. Immediately after the dye solution was injected, the epithelium showed diameters of one and three divisions of the eyepiece. Fifteen minutes later, when precipitation had begun to occur and the depth of color in the lumina had greatly increased, these same tubules showed diameters of four and five divisions. Again 15 to 30 minutes later, when the tubular lumina were decreased to a very narrow, deeply colored line, the readings were three and four divisions. This would therefore tend to indicate that during the time the dye solutions in the distal convolutions were showing a marked increase in depth of color, the cells were increased in size, as if something, probably water and some dye, was being taken up into the epithelium.

*Studies of reabsorption in tubules poisoned with mercuric chloride.* If the tubular epithelium reabsorbs water and phenolphthalein, as is strongly indicated in these experiments, it should be possible to inhibit this reabsorption with some drug which decreases the function of the cells. It has been pointed out by Heilbrunn (1928) that weak concentrations of mercuric chloride produce a great increase in the viscosity of the protoplasm of the cells of Euglena and Arbacia. This action appears before the frank precipitation of protein from stronger solutions. No doubt this increase in viscosity will lead to decreased function. If reabsorption of water and some other substances is the primary function of the epithelium of the tubules, it should at least be inhibited by this metallic salt. Thus, the

work of Lepine and his students (1886–1912), and of Bainbridge, Collins and Menzies (1913), if shown to be correct, can undoubtedly be explained by this action. The study of the effects of mercuric chloride upon the functions of the tubules reported in this paper, was practically completed before the reports of the above named investigators were found. Accordingly, the effects of mercuric chloride upon reabsorption of water and dyes in the tubules and ureter of the frog's kidney, has been studied. Solutions of this salt in concentrations of from 0.25 to 0.000001 per cent have been injected at pressures of from 20 to 30 cm. of $H_2O$ for one minute. In most cases, immediately after the injection, 0.2 to 0.4 cc. of 10 per cent $Na_2SO_4$ plus 0.2 to 0.5 cc. NaCl have been injected intravenously to increase the formation of urine and wash out the excess mercury salt and thus prevent it from reaching the glomeruli. With equal numbers of active glomeruli in the two kidneys (counted on the ventral surface), or with fewer active glomeruli in the poisoned kidney, the latter always excreted a greater volume, usually double, and sometimes treble the amount of the normal kidney. Where this represents only a small percentage of the total glomeruli, these counts give a fairly satisfactory estimate of the glomerular activity of the kidney. These findings thus confirm the observations of Lepine and his co-workers upon the decreased ability of the tubules poisoned with mercury salts to reabsorb water.

The ability of the distal convoluted tubules to concentrate dye solutions injected by means of the ureter, was next determined. The tubules in the left kidneys of four frogs were injured with injections of 0.001 to 0.0001 per cent mercuric chloride at 25 cm. of $H_2O$ pressure for one minute. Ten to twenty minutes were then allowed to elapse in order to allow the kidney to wash out most of the mercury salt solution, following which sodium indigosulfonate or indigo-carmine solutions of 0.25 per cent were injected into these tubules. The microscope showed an injection of almost all of the distal convolutions. In about half of the tubules, a concentration of the dye solution was to be observed, requiring about twice as long a time as in the normal tubules, to produce the same degree of concentration. In many of the tubules there appeared to be very little change in the depth of color in one to one and one-half hours. Precipitation to the dye occurred, but its onset instead of being around 5 to 10 minutes, as in the normal, was around 25 to 35 minutes, and it was present in the majority, at from one to one and one-half hours, again instead of 25 minutes in the normal. *This is undoubtedly associated with decreased water reabsorption.* When precipitation did occur in the tubules of the poisoned kidney, it appeared very much like that observed in the normal kidney.

The reabsorption of phenolsulphonephthalein from tubules and ureter poisoned with $HgCl_2$ with resulting excretion by the opposite kidney, was next studied, and the results were compared with the normal. Table 1

TABLE 1

*Excretion of phenolsulphonephthalein by normal right kidneys following reabsorption of the dye from the tubules of normal and poisoned left kidneys*

| EXCRETION BY NORMAL RIGHT KIDNEY FOLLOWING REABSORPTION FROM NORMAL LEFT KIDNEY | | | | | EXCRETION BY NORMAL RIGHT KIDNEY FOLLOWING REABSORPTION FROM INJURED LEFT KIDNEY | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Frog number | Left kidney injections | Right kidney dye excretion | | | Frog number | Left kidney injections | Right kidney dye excretion | | |
| | | First appearance | Time of excretion | Amount of dye | | | First appearance | Time of excretion | Amount of dye |
| | | *minutes* | *hours* | *per cent* | | | *minutes* | *hours* | *per cent* |
| 1 | 0.4 per cent PSP at 25 cm. $H_2O$ pressure for 1 minute | 4 | 1 | 300 | 2 | 0.00001 per cent $HgCl_2$ at 25 cm. $H_2O$ pressure for 1 minute; then 0.4 per cent PSP at 20 cm. pressure for 1 minute | 10 | 1 | 100 |
| 3 | Same at 20 cm. pressure | 6 | 1 | 400 | 4 | 0.00001 per cent $HgCl_2$ at 25 cm. $H_2O$ pressure for 1 minute; then 0.4 per cent PSP at 20 cm. pressure for 1 minute | 12 | 1 | 100 |
| 5 | Same at 20 cm. pressure | 5 | 1 | 600 | 6 | 0.0001 per cent $HgCl_2$ at 25 cm. $H_2O$ pressure for 1 minute; then 0.4 per cent PSP at 20 cm. pressure for 1 minute | 25 | 1 | 200 |
| | | | | | 7 | 0.0001 per cent $HgCl_2$ at 25 cm. $H_2O$ pressure for 1 minute; then 0.4 per cent PSP at 20 cm. pressure for 1 minute | 30 | 1 | 100 |
| | | | | | 8 | 0.0001 per cent $HgCl_2$ at 25 cm. $H_2O$ pressure for 1 minute; then 0.4 per cent PSP at 20 cm. pressure for 1 minute | 18 | 1 | 25 |

Urines of 1 and 2, and 3 and 4 were practically equal.

Urines of 6, 7 and 8 were one-half to two-thirds the volume of no. 5.

shows the results obtained in eight frogs. In frogs 1, 3 and 5, the study involved only normal kidneys. Into the left ureters, phenolsulphonephthalein was injected at pressures of 20 and 25 cm. $H_2O$ for one minute. Following the first appearance of the dye in the urine from the opposite kidney, the urine containing the dye was saved for a period of one hour.

In frogs 2, 4, 5, 7 and 8, the left kidneys were injured with $HgCl_2$ before the administration of phenolsulphonephthalein. In the first two frogs, 0.00001 per cent $HgCl_2$ was injected, and in the last three, 0.0001 per cent $HgCl_2$. Following the injection, 10 to 20 minutes were allowed to elapse during which time the injured kidney produced urine to wash out as much of the excess mercury as possible. In frogs 2 and 4, immediately after the exposure to mercury, and in frogs 1 and 3 after the dye injection into the ureter, from 0.2 to 0.3 cc. of $Na_2SO_4$ 10 per cent, plus 0.2 to 0.3 cc. of saline, was injected intravenously to increase the production of urine. In the remaining frogs no injections were made. Following this, 0.4 per cent phenolsulphonephthalein was injected into the injured tubules.

As can be seen from the table, the tubules and the ureter of the left normal kidney, frog 1, were able to reabsorb enough dye to result in an excretion by the opposite kidney three times as great as that obtained in the frog reabsorbing the dye by means of poisoned tubules and ureter (frog 2). But the urine volumes in both cases were almost equal. The same experimental conditions held for frogs 3 (normal left kidney) and 4 (poisoned left kidney). Here the normal kidney reabsorbed sufficient dye to result in an excretion by the opposite kidney four times as great as that excreted by the frog reabsorbing the dye in the same way by means of poisoned tubules. Finally the same results were obtained in the remaining frogs of the series. Frog 5 excreted an amount of dye in its right urine, following reabsorption of the dye in the tubules of the left normal kidney, equal to 600 per cent, whereas in the three other frogs with injured tubules and ureter, the amount of dye reabsorbed resulted in excretions by the opposite or right kidneys equal to 200, 100 and 25 per cent, respectively.

Thus it can be seen again that the normal tubules together with the ureters can reabsorb a fairly large amount of phenolsulphonephthalein into the blood stream, part of which is then excreted by the opposite kidney. In these frogs the dye appeared first on the opposite side on an average of five minutes. In the other experiments where the tubules were first poisoned with mercuric chloride, the reabsorption was much slower and less in amount. It required, on an average, 19 minutes for the first appearance, and following this the amount excreted during one hour was at the most only one-third as great as that contained in the urines from the above frogs.

If mercuric chloride thus inhibits the reabsorption of water and phenol-

sulphonephthalein in the tubules of the frog's kidney, the study of their excretion by the two kidneys of the same animal, one normal and one having tubules injured with mercuric chloride, should show the same findings. This has been the case in the series of frogs studied, providing one takes into consideration the number of glomeruli in each kidney showing active flow of blood. As has been shown by Bieter (1929) the injection of irritants into the ureter and tubules of the frog's kidney produces what appears to be a reflex inhibition of glomerular capillary blood flow. This same phenomenon has been observed here and, as a result, the injured kidney has in most instances shown fewer active glomeruli than the opposite or normal kidney. Possible swelling of the tubular epithelium following the application of mercuric chloride must also be considered. This has undoubtedly played a rôle in the use of the stronger concentrations, but in the others it appears to be of less importance.

Solutions of mercuric chloride varying in concentration from 0.25 to 0.0000001 per cent have been used. These solutions have been injected into the left ureter, as previously described. From experience, it has been found beneficial to inject from 0.1 to 0.4 cc. of $Na_2SO_4$ 10 per cent solution plus 0.2 to 0.4 cc. saline intravenously to enhance the production of urine. In the case of the kidney exposed to 0.25 per cent $HgCl_2$, at the end of an hour this kidney was markedly cyanotic and even brownish in places. The glomeruli were markedly shrunken and showed practically no blood flow and very little stasis. In kidneys where 0.1 to 0.01 per cent $HgCl_2$ was used, there appeared to be swelling of the tubular epithelium. The glomeruli again appeared more or less as above. Sections made of these kidneys and compared with normals showed in some areas tubular epithelium markedly swollen. The glomeruli appeared unaffected. Other sections of kidneys exposed to this same concentration of $HgCl_2$ were examined by Terry's method (1928) using scarlet red for stain. These sections showed that the tubular epithelium in many cases, especially of the distal convolutions, stained red, whereas normal kidney sections refused almost completely to take up the dye. Sections of kidneys exposed to $HgCl_2$ in concentrations of 0.0001 to 0.00001 per cent, have shown nothing abnormal. In some tubules there appeared to be increased vacuole formation in the cells, but this has not been a constant finding. Thus, while the evidence at hand does not show a marked and constant change in the structure of the cells of the tubules, there are sufficient indications to show that they exist.

The study of albuminuria in these frogs is also interesting. Using $HgCl_2$ in concentrations from 0.25 to 0.001 per cent albumin in the urine, determined by the nitric acid test, has been a constant finding in the first thirty minutes after the exposure to the mercury salt. Following this interval it tends to decrease. With concentrations of $HgCl_2$ from 0.0001

to 0.0000001 per cent, on the other hand, positive albumin tests from the urine have been rare. This again would tend to indicate changes in the epithelium—greatest where the stronger solutions have been used. These findings furthermore would indicate that albuminuria, at least in the frog, may be tubular in origin.

Table 2 shows the results obtained in a series of frogs, the left kidneys of which were first injured with mercuric chloride in the per cent solutions described. The phenolsulphonephthalein solution containing 0.5 mgm. of the drug was injected intravenously as soon after the $HgCl_2$ application as it was determined that the two kidneys were responding with satisfactory excretions of urine. The time of the injection was noted and then

TABLE 2

*The excretion of water and phenolsulphonephthalein in the frog, by the right normal kidneys and by the left kidneys whose tubules and ureter were injured with mercuric chloride injected through the ureters*

| FROG NUMBER | CONCENTRATION OF $HgCl_2$ AT 25 CM. $H_2O$ PRESSURE FOR 1 MINUTE | INJURED KIDNEY | | | | | | | NORMAL KIDNEY | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Appearance PSP | Urine excreted | | | | | | Appearance PSP | Urine excreted | | | | | |
| | | | After 10 minutes | | After 20 minutes | | After 30 minutes | | | After 10 minutes | | After 20 minutes | | After 30 minutes | |
| | | | Volume | PSP | Volume | PSP | Volume | PSP | | Volume | PSP | Volume | PSP | Volume | PSP |
| | | *minutes* | *per cent* | *per cent* | *per cent* | *per cent* | *per cent* | *per cent* | *minutes* | *per cent* | *per cent* | *per cent* | *per cent* | *per cent* | *per cent* |
| 1 | 0.25 | 2 | 100 | 100 | 100 | 100 | 50 | 100 | 3.5 | 100 | 100 | 150 | 400 | 250 | 600 |
| 2 | 0.1 | 2 | 100 | 100 | 100 | 100 | 50 | 100 | 2.5 | 100 | 100 | 150 | 200 | 150 | 400 |
| 3 | 0.01 | 3 | 200 | 100 | 200 | 100 | 200 | 100 | 5.5 | 100 | 100 | 100 | 100 | 100 | 100 |
| 4 | 0.001 | 2 | 100 | 100 | 100 | 200 | 100 | 100 | 4 | 100 | 100 | 100 | 100 | 100 | 100 |
| 5 | 0.0001 | 4 | 100 | 100 | 100 | 100 | 100 | 100 | 5 | 50 | 100 | 100 | 50 | 100 | 100 |
| 6 | 0.00001 | 2.5 | 125 | 125 | 150 | 125 | 150 | 125 | 3.5 | 100 | 100 | 125 | 100 | 125 | 100 |
| 7 | 0.000001 | 2 | 120 | 100 | 120 | 120 | 100 | 100 | 2.5 | 100 | 100 | 100 | 100 | 100 | 100 |
| 8 | 0.0000001 | 6.5 | 125 | 100 | 125 | 400 | 125 | 300 | 7 | 100 | 100 | 100 | 300 | 100 | 300 |

the presence of the dye in the urines in the cannulae was noted. In all cases the dye appeared in the urine coming from the injured kidney before it appeared in the urine from the normal kidney. The times for each experiment are recorded in the tables. They show an average of three minutes for the appearance of the dye in the urine from the injured kidney and 4.2 minutes from the normal kidney. This tends to show that the first dye in the glomerular filtrate was reabsorbed by the tubular epithelium and thus did not appear in the urine as soon as it did on the side with poisoned tubules. After the first few minutes of excretion by the kidneys injured with $HgCl_2$ 0.25 to 0.01 per cent, the urine volume tended to decrease and along with it the amount of dye. Thus from this point on, the

opposite or normal kidney showed a greater excretion of both dye and water. On the other hand, the kidneys exposed to $HgCl_2$ in concentrations of from 0.001 to 0.00001 per cent, did not show this change. The urine volume from the first, in most instances, was greater than that from the opposite or normal kidney and likewise the excretion of phenolsulphonephthalein. A number of frogs have been subjected to each of the above concentrations with essentially the same findings. Using concentrations of $HgCl_2$ of 0.000001 per cent and less, the inhibition of reabsorption has not been as great as with the slightly stronger concentrations.

In all of the experiments, the injured kidney has not excreted more water and more dye than the normal kidney. The answer to this variation

TABLE 3

*The relation of phenolsulphonephthalein excretion in the frog to glomerular blood flow in normal kidneys and in kidneys with tubules poisoned with mercuric chloride injected through the ureters*

| FROG NUMBER | CONCENTRATION OF $HgCl_2$ INJECTED INTO LEFT URETER | INJURED LEFT KIDNEY | | NORMAL RIGHT KIDNEY | |
|---|---|---|---|---|---|
| | | Number of active glomeruli | Amount PSP excreted (10-minute interval) | Number of active glomeruli | Amount PSP excreted (same 10-minute interval) |
| | | | *per cent* | | *per cent* |
| 1 | 0.25 | 6 | 100 | 54 | 600 |
| 2 | 0.1 | 15 | 100 | 45 | 200 |
| 3 | 0.01 | 30 | 100 | 42 | 100 |
| 4 | 0.001 | 35 | 100 | 46 | 100 |
| 5 | 0.0001 | 32 | 100 | 68 | 100 |
| 5a | 0.0001 | 63 | 110 | 85 | 100 |
| 6 | 0.00001 | 36 | 125 | 41 | 100 |
| 6a | 0.00001 | 38 | 110 | 76 | 100 |
| 6b | 0.00001 | 35 | 125 | 50 | 100 |
| 7 | 0.000001 | 46 | 120 | 30 | 100 |
| 8 | 0.0000001 | 48 | 100 | 55 | 100 |

appears to be the number of glomeruli per kidney showing active blood flow, and is shown in table 3. The numbers at the left of the table indicate the frogs of the same number shown in table 2, with the addition that several more experiments on separate kidneys for concentrations of $HgCl_2$ of 0.0001 and 0.00001 per cent are included.

From this table it can be seen that in frog 1 the injured kidney showed very few active glomeruli upon its ventral surface, while the opposite kidney showed 54. The injured kidney eliminated only one-sixth as much dye and water as the normal kidney during this time, showing the relation between glomeruli with active blood flow and urine formation. A similar difference in excretion is shown again in frog 2. In frogs 3, 4 and 5, the

number of active glomeruli seen in the injured kidney amounted to 30, 35 and 38, while the normal kidneys showed 40, 46 and 68, respectively. The injured kidneys of this series put out just as much or slightly more urine, and just as much dye, as the normal kidneys. In other words, 34 active glomeruli, on an average, with tubules poisoned with $HgCl_2$ were able to excrete as much urine and dye as 51 glomeruli, on an average, with normal tubules under the conditions of these experiments. Turning now to frogs 5a, 6, 6a and 6b we find: 63, 36, 38 and 35 visible active glomeruli in the injured kidneys, and 85, 41, 76 and 50 visible active glomeruli in the normal kidneys. Averaging these figures, we find that 45 active glomeruli with injured tubules were able to excrete slightly more dye and urine than 63 active glomeruli with normal tubules. Lastly, in frogs 7 and 8, the $HgCl_2$ concentrations used were apparently not sufficient to inhibit the tubular activity. In a previous communication (1929) it has been shown that the frog's kidney excretes phenolsulphonephthalein rapidly and in large amounts when it is injected via the renal portal vein, but only when the renal portal blood containing the dye reaches the glomerular capillaries. *Therefore, the experiments herein reported indicate, 1, that after the first and rapid elimination of phenolsulphonephthalein by the glomeruli, a certain portion of the dye is reabsorbed by the epithelium of the tubules, and 2, that when this reabsorptive function of the tubular epithelium is inhibited by means of $HgCl_2$ injected into the tubules through the ureter, equal numbers of active glomeruli with poisoned tubules can excrete more water and dye than an equal number of glomeruli with normal tubules.*

These experiments furthermore tend to raise a doubt as to the interpretations of studies on the excretion of phenolsulphonephthalein reported by Edwards and Marshall (1924), Marshall and Crane (1924) and Crane (1927), which can also be explained by the reabsorption of the dye from the lumen as has been shown above to occur. In like manner, the interpretations of Starling and Verney (1925) upon the decrease in excretion of phenolsulphonephthalein produced in the isolated kidney by the addition of cyanide, must now be questioned. For the acceptance of their conclusions, it must be shown conclusively that the cyanide has no effect upon the elimination of the dye through the glomerular capillaries.

Lastly, the clinical observations of Prof. George Fahr in nephrosis are in complete agreement with the experimental findings of this paper. In this condition the tubules represent the parts chiefly affected, and thus they perhaps show a decreased function. In the use of Rowntree and Geraghty's phenolsulphonephthalein test in his cases of nephroses, Doctor Fahr has found an increase in the elimination of the dye, as well as of fluid, over the normal.

The excretion of sodium indigosulphonate by the two kidneys of the same frog, one normal and one with the tubules injured with mercuric

chloride, from 0.0001 to 0.00001 per cent, was now studied. Because this dye has been shown to be reabsorbed by the tubules to a much less extent than phenolsulphonephthalein, one would expect little change in the elimination of this dye by the injured kidney, which has been found to be the case. The results obtained are shown in table 4. In frog 4, 39 visible, active glomeruli in the kidney with injured tubules excrete 150 per cent as much urine as 36 visible active glomeruli in the normal kidney. In like manner, in frog 1, 25 active glomeruli in the injured kidney excrete a urine volume equal to that eliminated by 56 active glomeruli in the normal kidney. The same variations are to be observed in the other frogs. The total dye eliminations, however, during intervals of from 10 to 30 minutes, do not show these same variations. Thus in frog 4 with equal numbers of

TABLE 4

*The excretion of sodium indigosulfonate and water in the frog, by right normal kidney, and by left kidney with tubules injured with mercuric chloride, injected through the ureter, and its relation to glomerular blood flow*

| FROG NUMBER | CONCENTRATION OF $HCl_2$ USED | DURATION OF EXCRETION | INJURED LEFT KIDNEY | | | NORMAL RIGHT KIDNEY | | |
|---|---|---|---|---|---|---|---|---|
| | | | Active glomeruli | Amount of dye excreted | Amount of urine excreted | Active glomeruli | Amount of dye excreted | Amount of urine excreted |
| | | *minutes* | | | | | | |
| 1 | 0.0001 | 15 | 26 | 50 | 100 | 59 | 100 | 100 |
| | | 15 | 25 | 50 | 100 | 54 | 100 | 100 |
| 2 | 0.0001 | 30 | 49 | 120 | 150 | 39 | 100 | 100 |
| | | 15 | 17 | 300 | 200 | 3 | 100 | 100 |
| 3 | 0.00001 | 10 | 54 | 90 | 90 | 64 | 100 | 100 |
| 4 | 0.00001 | 30 | 39 | 100 | 150 | 36 | 100 | 100 |
| 5 | 0.00001 | 30 | 23 | 85 | 120 | 35 | 100 | 100 |

Dyes appeared in urines 5 to 12 minutes after intravenous injections. Difference between the two sides not greater than 20 seconds.

visible active glomeruli in each kidney, the dye excretions are equal. In frog 1 with only 25 active glomeruli visible in the injured kidney, the dye excretion is only one-half of what it is in the normal kidney with 56 active glumeruli. The same relationship to glomerular blood flow is to be observed in the other frogs of the series. The dyes appeared in the urines of both the injured and normal kidneys from 5 to 12 minutes following the intravenous injections of 1 mgm. of the dye in 0.5 cc. of saline, and the appearances in the two urines of the same frog were never more than 20 seconds apart.

## CONCLUSIONS

1. A technique of injecting various substances, by means of the ureter, into the tubules of the frog's kidney for the purpose of studying reabsorp-

tion, is herein described. Using Richards' method of direct observation, changes produced in the solutions in the lumina of the tubules and in the epithelium can be noted.

2. As pyelovenous backflow does not occur within the normal frog's kidney, it is thought to be safe to use this method for intratubular injections.

3. Phenolsulphonephthalein when injected into the proximal and distal convoluted tubules in the normal frog's kidney is reabsorbed. At least a part of this reabsorbed dye is excreted by the opposite kidney.

4. Phenolsulphonephthalein when injected into the ureter and larger collecting ducts also shows this reabsorption and resulting excretion by the opposite kidney, but to a much less extent.

5. Sodium indigosulphonate, indigo-carmine and trypan blue, injected in a similar manner, are only reabsorbed to a very slight degree.

6. When a solution of one of the above dyes is injected into the distal convolutions of the frog's kidney, concentration of these solutions begins immediately. Precipitation of the dye particles within the tubular lumen from solutions of from 0.25 to 0.05 per cent begins in from 5 to 20 minutes. With solutions of 0.25 per cent the precipitation is almost complete in 20 to 30 minutes. This undoubtedly occurs primarily from reabsorption of water.

7. During this process of concentration, the diameter of the tubular epithelium appears increased, as if the cells were taking up some substance to increase their bulk.

8. Mercuric chloride in dilute solutions inhibits the concentration of the dyes within the lumen of the tubules described above. Following the application of this salt, precipitation from a solution of 0.25 per cent begins in 25 to 35 minutes and in some cases is not complete in 90 minutes.

9. Mercuric chloride again injected into the convoluted tubules of the frog's kidney produces a marked inhibition of what appears to be reabsorption of water and phenolsulphonephthalein.

10. The inhibition of tubular reabsorption of both water and dyes produced by mercuric chloride tend to confirm the findings of Lepine and his students, carried out on mammalian kidneys, and possibly also those of Bainbridge, Collins and Menzies obtained through a different route.

11. The results obtained in these experiments in the frog's kidney fit into and support Cushny's "Modern theory of kidney function" more satisfactorily than they do any of the other common theories of kidney function.

I wish to express my sincere appreciation to Dr. A. D. Hirschfelder under whose direction the work was completed, for helpful suggestions and criticisms.

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# MECHANICAL FACTORS CONTRIBUTING TO THE EXCHANGE OF FLUIDS IN THE BODY

## I. Conditions Favorable to Ejection, Filtration, Injection, Aspiration and Deflection of Fluids through Tubes and Membranes

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Several of the special problems which will be considered in the papers of this series developed mainly out of some rather perplexing observations made by me on animals in health and disease in some large zoölogical gardens, principally the Hagenbeck Animal Park in Stelligen, the Berlin Zoölogical Garden and the Washington Park Zoölogical Garden in Milwaukee. Due to the hearty coöperation of the persons most concerned with the welfare of the animals in the garden last named, a special opportunity has been afforded me in recent years to investigate carefully some of the facts which were earlier so disconcerting and to relate these with one another and with the more usual facts of human physiology and pathology.

My own efforts to explain fluid exchanges in the body in terms principally of osmosis and diffusion were in cases so obviously futile that a careful search was made for some auxiliary factors which might be operating—at least under certain conditions of circulation and respiration—in heretofore unsuspected or neglected ways. As a result of this search, my interest became centered on some blood vessels and certain other mechanisms of seemingly appropriate configurations to cause ejection, filtration, injection, aspiration and deflection to occur more or less separately in some instances but generally as dependent processes in the body. Some of the configurations referred to are permanent and some are transient.

Ejection, filtration, injection, aspiration and deflection may be considered as synonymous terms in so far as they all refer to movements of simple or complex fluids from areas of high to areas of low pressure, but it is possible and undoubtedly worth while to point out certain differences of meaning between them.

Air is ejected from the lungs, i.e., forced out of them, when the chest walls react to cause the air pressure in the lungs to exceed the pressure of the air outside the body. Blood is normally ejected from the aorta through the mouths of many of its branches. Blood may also be ejected

from the capillaries through the porous walls of these blood vessels, but if the red blood cells and perhaps some of the blood colloids are held back by the membranes, the porous capillary walls, this form of ejection is filtration out of the capillaries and the escaped fluid is the filtrate.

Injection is the reverse of ejection; the lungs, for instance, of a living or dead animal may be artificially inflated by injecting, i.e., forcing, a liquid or gas into them through the trachea. Blood may be injected into an artery through an anastomosis connecting this artery with another one in which the blood pressure is relatively high. A fluid may be under such great pressure in the tissue spaces that it is forced through the porous walls of some capillaries, but if some of the constituents of the tissue fluid are held back by the membranes, this form of injection is filtration into the capillaries and the fluid that passes is the filtrate.

Aspiration is a kind of injection, but it depends upon a diminished fluid pressure in such structures as the lungs or blood vessels; fluid may be aspirated into the lungs, as during a normal expansion of the chest, or into an artery, as when this is subjected to a sudden linear extension (as when the lungs inflate or the diaphragm relaxes or the abdomen expands) without deforming it in any way other than increasing its length and decreasing its lumen. If the blood enters the artery through the mouths of some or all of its branches, we should speak of aspiration only. If a capillary is suddenly stretched and the porous walls permit only certain constituents of an outside fluid to enter the capillary, this form of aspiration is filtration into the capillary and the fluid that enters is the filtrate.

Deflection in the body may depend upon ejection, filtration, injection and aspiration, but it may be thought of as being essentially a kind of aspiration through tubes or porous membranes because of a low pressure area created, in such a structure as a blood vessel, by a stream of fluid. The term deflection will refer primarily to such kinds of lateral fluid movement as may be obtained with the simple mechanical devices commonly known to hydraulicians as piezometer and Venturi tubes.

The present series of papers concerns the special mechanisms in the body that serve to produce differences in fluid pressure which are required for ejection, filtration, injection, aspiration, piezo action and Venturi action. In the present paper, which is introductory in nature, special attention will be given to the systemic arteries and their fine processes. Discussions of artificial devices that will be entered into will serve the purpose of showing how similar devices in the body must function.

Piezometric circulations. Figure 1 illustrates principally some effects that may be obtained with four piezometer tubes, *A*, *B*, *C* and *D*, placed at 45, 90, 135 and 90 degree angles respectively relative to the advancing stream in the tube *MN*. Only the solid lines should be considered at present. The length of the shorter piezometer tube, *D*, is

equal only to the thickness of the wall of $MN$. It is assumed that the pressure and rate of flow in $MN$ are such, relative to the external fluid pressure, that water stands at the indicated levels in $A$ and $B$ and that $C$ overflows. Of course water escapes from $D$ as it would from $B$ if $B$ were made as short as $D$.

It may be further assumed that the head of pressure that causes the flow in $MN$ is such that either the external fluid pressure (the lateral pressure, at the free ends of $A$, $B$, $C$ and $D$) or the rate of flow in $MN$ may be appropriately decreased to cause water to escape from $A$ and $B$ as well as

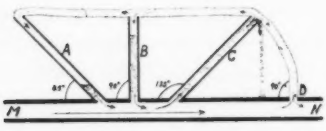


Fig. 1

Fig. 2

Fig. 1. A system of tubes, comparable to some arteries and their anastomoses, to indicate piezometric pressure differences and piezometric circulations that may be obtained by properly regulating either the pressure and rate of flow of water in the horizontal tube $MN$ or the lateral pressure or both. The arrows with solid shafts in the piezometer tubes $C$ and $D$ indicate the escape of water from $MN$ when $C$ and $D$ are permitted to overflow. The arrows with broken shafts indicate the directions of the piezometric circulations when the pressure at the distal ends of $A$, $B$ and $D$ is increased by connecting $A$, $B$, $C$ and $D$ by means of the tube drawn in dotted lines. The only condition under which the pressure can be the same in $A$, $B$, $C$ and $D$ is that there is absolute stasis of the liquid in the entire system.

Fig. 2. A network of blood vessels very much like that of an intestinal villus. Found in mesentery just outside the large intestine of a rhesus monkey. Specimen did not photograph clearly because of other blood vessels above and below it. Drawing made with camera lucida. A second network, to the left, is incompletely drawn. The arrows indicate the probable directions of the blood flow.

from $C$ and $D$. On the other hand, either the external pressure or the rate of flow in $MN$ may be appropriately increased to cause deflection into $MN$ through $A$, $B$ and $D$. Although the ejection or positive piezometric pressure in $C$ can be altered by altering the rate of flow in $MN$, an external fluid will not actually pass into $MN$ through $C$ unless the external pressure is made so great that we should speak essentially of injection instead of deflection through $C$ into $MN$.

One means of increasing the external pressure at the free ends of $A$,

*B* and *D* in figure 1 is to connect *A*, *B*, *C* and *D* by means of a special tube indicated by the dotted lines. The overflow from *C* will now be deflected into *MN* via *A*, *B* and *D* at rates depending upon the relative pressures in *A*, *B* and *D*. Neglecting the hydraulic gradient from *B* to *D* (the line to which the water would rise in vertical piezometer tubes at *B* and *D*) and a greater ease of spill into *D* than into *B* (in part a matter of hydraulic gradient also), the rate of deflection from *B* should be equal to that from *D* and less than that from *A*. The circulation of water from *C* back into *MN* via *A*, *B* or *D* is a piezometric circulation which is in all essential respects like many piezometric circulations in the body, as the circulation of blood from the arch of the aorta to the descending thoracic aorta via an internal mammary artery and any one of the several ascending aortic intercostal arteries. (These piezometric circulations in the body will be given special attention in some later papers.) A piezometric circulation may be influenced by but is not necessarily dependent upon the action of gravity. For instance, if *MN* were rotated through an angle of 180 degrees, hydrostatic and hydrodynamic pressure differences would still exist in *A*, *B*, *C* and *D*.

In making models on the plan of figure 1 it was found that the piezometric circulations were more readily obtained when *MN* was extended a considerable distance beyond *N* or when a resistance, as a constriction, was introduced to the right of *N*. In other words, a certain peripheral resistance favors strong piezometric circulations in such models, evidently because a peripheral resistance causes a relatively much greater increase in the piezometric pressure in *C* than in *A*, *B* or *D*. Such a peripheral resistance probably acts in the same way as many of the peripheral arterial anastomoses from which the blood can escape to the veins through the capillaries only or at least primarily (fig. 3). The peripheral resistance is probably at times optimal but at other times either too high or too low to permit the strongest piezometric circulations to occur in some of the configurations of arteries in which piezometric circulations can occur.

As suggested by figures 2 to 8 inclusive, branches of large and small arterial trunks may be connected by anastomoses somewhat as is indicated in figure 1 by the tube drawn in dotted lines to connect *A*, *B*, *C* and *D* to permit deflection into *MN* in spite of the circumstance that the conditions of flow in *MN* were assumed to favor ejection rather than deflection. *MN* in figure 1 may be likened to the descending thoracic aorta, *A* to the aortic stump of an ascending aortic intercostal artery, *C* to the aortic stump of a descending aortic intercostal artery and the tube in dotted lines to the numerous large and small arterial highways uniting the aortic stumps (figs. 4, 5 and 6).

*A* and *C* of figure 1 may be likened, also, to the trunks of two arterial bushes that spring from the same or from two relatively large arterial

trunks (figs. 2, 3, 4, 5, 6 and 8). An arterial bush may consist of a more or less well-defined trunk and few or many branches or smaller arterial bushes which may be interconnected by anastomoses ranging in size from very small capillaries to relatively large arterial channels. If the piezometric pressure in *C* and its capillary anastomoses is greater than that in *A*

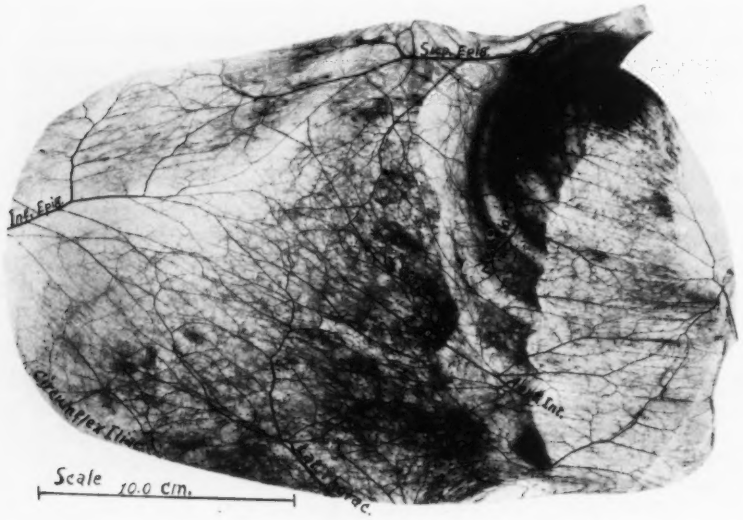


Fig. 3. Part of right abdominal wall and a part of the diaphragm of a 139 day old Bengal tiger injected with India ink and cleared. Some costal cartilages visible through diaphragm, peritoneum and transverse layer of abdominal muscle, the other muscle layers being cut away for a short distance below the cartilages. Blood in certain parts of a phrenic vein render it visible, but no vein is injected in the entire specimen. The arteries are very imperfectly injected, but specimens that were perfectly or almost perfectly injected showed up in the photographs as unanalyzable masses of black. *Inf. Epig.*, part of inferior epigastric artery; *Sup. Epig.*, superior epigastric artery; *Lat. Thorac.*, lateral thoracic artery; *Circumflex Iliac*, a few of the branches of the ascending branch of the circumflex iliac artery; *Aort. Int.*, one of the aortic intercostal arteries that does not anastomose conspicuously with the musculophrenic artery.

and its capillary anastomoses, fluid will tend to filter out of the capillaries of *C* and pass into those of *A*. If fluid actually passes through the tissue spaces and cells from one set of capillaries to another, this is a piezometric circulation independently of whether the fluid is then deflected farther back into the arterial circulation or is merely carried on into the veins. As a rule, two or more large or small neighboring arterial bushes are con-

nected by capillaries so that a piezometric circulation can take place through these small channels (as well as through larger anastomoses that are generally present) without having to leave one set of capillaries and circulate through the cells and cell spaces to a low pressure area in another set of capillaries.

Capillaries here and there in the body seem to unite a single arteriole with a single venule or the arterioles of a single arterial bush with the venules of a single venous bush, but it may require considerable patience to find such simple connections in many parts of a well-injected animal.

Fig. 4

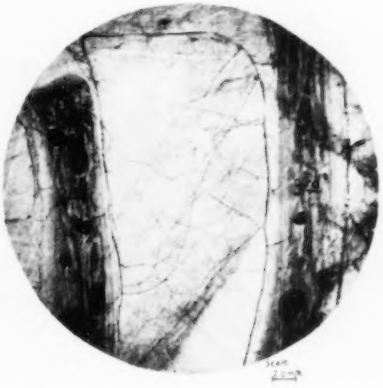

Fig. 5

Fig. 4. Aortic intercostal arteries at posterior margins of eighth, ninth and tenth ribs of human infant at birth. Only some of the larger anastomoses injected. No veins visible. Observe the angles that some of the branches form with the large arteries. Left chest wall.

Fig. 5. Arterial anastomoses in second intercostal space of an old rhesus monkey. No veins injected. Left chest wall.

It is very doubtful whether such simple connections exist in many parts of the body. A capillary anastomosis that connects two large arterial bushes or parts thereof with one another is usually a capillary network that also connects the arterioles of the arterial bushes with venules (fig. 2). In so far as such a network connects arterial bushes it is a terminal or heterocladic anastomosis, although the arterioles may also be connected to venules by the same network.

Generally speaking, a large heterocladic anastomosis is more than an arterial highway which tends to bring about an equalization of pressure in two arterial systems. It is also a principal arterial trunk, relative to the bushes that originate from it (figs. 2, 3, 6 and 8). A piezometric

circulation through such an anastomosis should be expected to play an important part in establishing piezometric circulations in the heterocladic capillary anastomoses of its arterial bushes. Piezometric circulations may be established in the capillary anastomoses because the trunks of the arterial bushes form different angles with the advancing stream in the large heterocladic anastomosis or because the bushes of this anastomosis

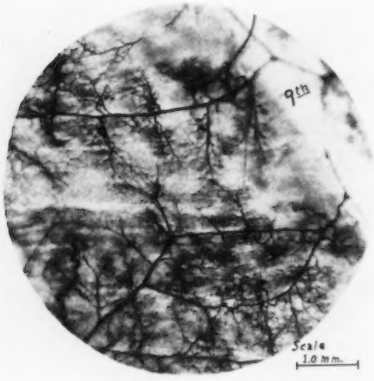


Fig. 6

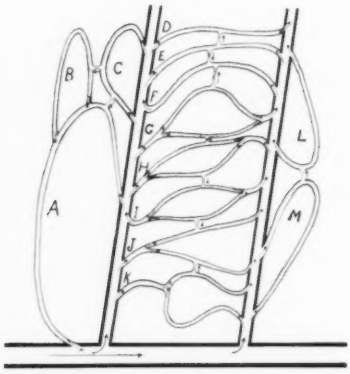


Fig. 7

Fig. 6. Arterial anastomoses in eighth intercostal space of an adult Wisconsin porcupine. The large anastomoses associate the eighth and ninth aortic intercostal arteries and these are associated by smaller arterial bushes and anastomoses which are in turn associated with one another by capillary and near-capillary anastomoses. No veins injected. Right chest wall.

Fig. 7. Representation of some special configurations of arteries that may be found here and there in almost any part of the body. *C*, *E*, *H*, *M* and a part of *K* are obviously pressure or filtering stations and *I* and *L* are obviously suction stations. The only condition under which the liquid pressure could be the same in all the configurations is that absolute stasis exists throughout the system. The arrows indicate the directions of flow at various points in the system when the flow is from left to right in the horizontal pipe. The small necks connecting the configurations represent connections or leaks from one to another similar to many found between many arterial anastomoses in the body. These necks are also intended to represent Venturi tubes, such as shown in figure 9.

connect with bushes that originate at such angles from another one that the pressure is either relatively low or relatively high in the bushes of the second large anastomosis. Some reasons for differences of pressure in different large anastomoses uniting the trunks of two large arterial bushes are indicated in the scheme presented as figure 7. The only reasonable condition under which the fluid pressure can be the same in all of the anastomoses represented in this figure is that there is complete stasis of

fluid in the entire system. Obviously, the same is true of the numerous, similar anastomoses in the body. Any variation in the arterial circulation must mean a change of pressure and rate of flow of blood in at least some of the arterial anastomoses and accordingly a reversal here and there in the direction of flow in some of the small anastomoses of the larger ones. Such variations in the arterial circulation are the velocity and pressure pulses, rhythmic fluctuations due to the respirations, rhythmic and arthymic fluctuations due to reactions of muscles, variations due to general changes in heart action and various fluctuations due to local and general changes in caliber of the arterioles and capillaries.

Excessively high arterial blood pressure should tend to reduce deflection in some of the arterial bushes and anastomoses to a minimum, an irreducible minimum; it might become negligible in some but not in all of the bushes and anastomoses. As a guard, it seems, against excessive stasis, excessive pressure, excessive filtration and excessive accumulation of blood cells in any anastomosis, "leaks" from one anastomosis to another (as well as to veins) exist, as indicated in figure 7 and as shown in figure 6. The circulation through one of the leaks from one anastomosis to another may itself be a piezometric circulation which may become greatly augmented, rather than diminished, when the arterial blood pressure becomes excessively high.

Some of the leaks herein considered are capillaries that connect arterial bushes and anastomoses with veins. Those associating the arterial bushes and anastomoses with one another range in size from very small capillaries to relatively large arterial channels. None of the leaks here discussed should be confused with the "derivating canals" or "arteriovenous anastomoses" reported by Hoyer (1877) and Heimberger (1925). These canals are described as blood vessels of considerable size, relative to capillaries, that connect arteries or arterioles directly with venules or veins. I have been very unfortunate in my efforts to find such canals in animals that were injected either before or after death. There may be some unknown reason or reasons, however, why these canals should not become injected at least as well as capillaries.

It is important that the piezometric circulations involve capillaries, for the principal biological purposes of these circulations probably are: Blood is caused to linger in capillaries and therefore have extra time to give up its nutritive substances and to take up products of catabolism; a filtrate of the blood is permitted to circulate through the tissue cells and spaces and accordingly give up its nutritive substances and take on waste products somewhat better than if it remained in the capillaries; the acidified fluid that passes in instances back into the large arteries reduces the stability of the oxyhemoglobin compound so that freshly aerated blood becomes contaminated and thereby prepared to give up its oxygen even before it

reaches the capillary channels to pass over into the veins; a rich circulation of adequately acidified arterial blood is supplied to certain cells that ordinarily function without much rest, as the cells of the intercostal muscles. The effect here referred to of an acid medium on the stability of the oxyhemoglobin compound was pointed out by Bohr, Hasselbalch and Krogh (1904).

The traditional notions concerning the biological purposes of arterial anastomoses are expressed quite well by Kimber and Gray (1925) as follows: "Such inosculations admit of free communication between the currents of the blood, tend to obviate the effects of local interruption, and to promote equality of distribution and of pressure." According to the reasoning followed in this paper, at least many of the anastomoses of the systemic arterial network tend to promote inequality rather than equality of pressure.

VENTURI ACTION. Piezometric deflections are evidently greatly augmented at numerous points in the body by necks or constrictions in small blood vessels. These constrictions are the necks of Venturi tubes. They are illustrated in figure 7 by the necks connecting various anastomoses, they are shown in figure 6 as necks of various lengths connecting some arterial bushes and they are also shown in figure 8 as constricted portions of an artery, at each end of a physiological aneurism.

A Venturi tube used by hydraulic engineers as an essential part of a flow meter (a Venturi meter) is illustrated in figure 9. The Venturi meter preferred as standard is one which consists essentially of frustrums of two cones, *MO* and *NO*, and of two vertical piezometer tubes, *A* and *B* (fig. 9). The frustrums have altitudes in the ratio of 1 to 3. The large bases of the frustrums are equal. The small bases are also equal and are joined to form the neck of the meter. Both piezometer tubes should have equal amounts of air extracted from them to prevent inconveniently strong deflection at the neck and to raise the water columns to convenient levels for making necessary readings. The difference in length of the water columns in the piezometer tubes of a calibrated meter are required for determining the flow of water in a pipe into which the meter is inserted in such a way that the stream advances into the short frustrum and retreats through the long one.

Venturi tubes may deviate considerably, however, from the preferred type and still deflect quite well. Since we are not especially interested in the device as a flow meter but as one for bringing about or merely augmenting deflection of fluids in the body, we should not consider it entirely essential that a piezometer tube in the neck has a length greater than the thickness of the wall of the neck, that the two frustrums have altitudes in the ratio of exactly 1 to 3 or that the piezometer tubes stand at right angles to the stream in the Venturi tube. The deflection might

be inconveniently great for purposes of determining the flow in pipes if *B* in figure 8 formed an acute angle with the advancing stream in the Venturi tube or if the neck of the meter were in an area of unusually high fluid pressure, but both of these conditions may exist in the body and serve the useful biological purpose of augmenting deflection into arteries.

In many instances the walls of the arterial Venturi tubes are evidently too thick and non-porous for fluid to pass through them except via certain

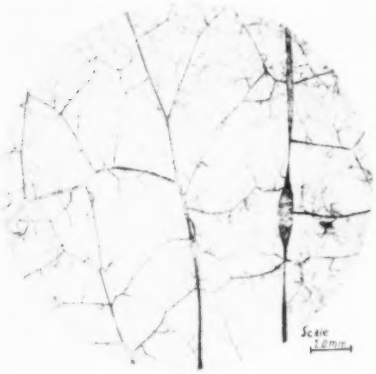


Fig. 8

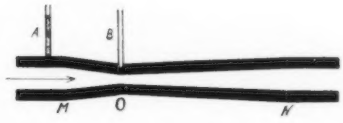


Fig. 9

Fig. 8. Temporary aneurisms and Venturi tubes in some small arteries in a wall of the large intestine of an adult capuchin monkey. A Venturi tube is at each end of the largest ballooned portion. Numerous ballooned portions were visible to the unaided eye, but only a small number could be found after the injection was made and the specimen cleared. The intestines were still active when the injection was made. No veins injected.

Fig. 9. A Venturi tube such as is preferred by hydraulic engineers as a flow meter. It consists essentially of the frustrums of two cones, *MO* and *NO*, and of two vertical piezometer tubes, *A* and *B*. The only condition under which the piezometric levels in *A* and *B* can be the same is that there is absolute stasis of fluid in the horizontal tube.

well-defined channels. These channels are the trunks of small arterial bushes which are generally present as lateral outgrowths from and near the necks of the larger Venturi tubes. These trunks are comparable to the piezometer tubes *A* and *B* in figure 9, but they often form various angles with the advancing streams in the Venturi tubes. There are various possibilities, therefore, of different pressure areas being created in and about the capillaries and other parts of the arterial bushes that originate from and near the neck of a Venturi tube, and the effect should be the same as if the walls of the Venturi tubes were a flimsy, porous meshwork.

The walls of some of the smaller Venturi tubes are probably porous in the sense that the piezometer tubes, instead of being trunks of arterial bushes, are short tubes equal in length only to the thickness of the walls of the respective Venturi tubes.

The walls of the temporary Venturi tubes at the ends of a temporary ballooning of a blood vessel, as shown in figure 8, are likewise porous in one or both of the senses just stated. The same is also true of the ballooned portions. This ballooning takes place in blood vessels ranging in size from capillaries to relatively large arteries. They have been observed in this laboratory to occur in the walls of the stomach and intestines of lightly anesthetized animals. The conditions under which the observations were made were such as to warrant the belief that the ballooning is a normal physiological process, although there is a possibility that it is excessive in some animals during the agony of death. I have succeeded in fixing and injecting some balloons in only three animals, in the large intestine of a capuchin monkey (fig. 8), in a portion of the peritoneum of a rhesus monkey and in the small intestine of a loon. The ballooning of any vessel, as observed either with the unaided eye or with a microscope and strong transmitted light, was rarely found to last longer than four or five seconds in animals that were anesthetized or that were dying, as after being shot through the head or chest. It is probably the case that filtration and even diapedesis occurs at a ballooned portion and that deflection occurs at the constricted portions at the ends of a balloon, as will be pointed out in a later paper, particularly in connection with petechial hemorrhages.

General discussion. The diverging system of blood vessels which we commonly speak of as the systemic arterial tree is an almost inconceivably complex network of arteries, arterioles and capillaries with a sufficient number of connections with venules to admit of an adequate return of blood to the heart via the systemic veins. An examination of the literature reveals that this concept is not entirely new. It is well known, for instance, that systemic capillaries generally form networks and that a single network may connect many arterioles with many venules. A point especially emphasized, however, in this paper is that any number of arterioles that are associated with any number of venules by a capillary network are themselves associated by the same network so that blood may flow principally from some of the arterioles into others or principally from the arterioles into their associated venules.

Spalteholz (1888, 1927) and others have shown that arterial anastomoses are quite numerous at various parts of the body, and some of the inosculations are considered briefly in our text books of anatomy; but it was not known, I believe, that the anastomoses are as numerous and diversified throughout the body as my best injected specimens show them to be.

It is also not entirely new that acidified blood may be thrown back,

as centripetal streams, into the arterial circulation. It is well known, for instance, that some of the blood that circulates in the walls of the heart passes centripetally into the left heart via the venae cordis minimae (Spalteholz, 1920), but adequate reasons have heretofore not been advanced, I feel, for believing that circus movements of blood can occur in many parts of the systemic arterial system of blood vessels.

## CONCLUSIONS

1. The diverging system of systemic blood vessels is an almost inconceivably complex network of arteries, arterioles and capillaries with a sufficient number of capillary connections with the converging system of systemic blood vessels to permit an adequate return of blood to the heart.

2. Observations herein reported, and alluded to, warrant the beliefs that circus movements of blood normally take place in a considerable number of configurations of arteries and that a circus movement in any of the diminutive arterial systems is due to differences of fluid pressure caused at different parts of the system by different degrees of piezo and Venturi action. Since Venturi tubes not provided with piezometer tubes would be useless in the body, all of the accessory arterial circulations are called piezometric circulations.

3. The piezometric circulations probably assist greatly in regulating the relative amounts of water in the blood vessels and tissues and in bringing about adequate exchanges of respiratory gases and other substances at the tissues.

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# MECHANICAL FACTORS CONTRIBUTING TO THE EXCHANGE OF FLUIDS IN THE BODY

## II. In the Lungs of the Mammal

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A few of the phenomena which undoubtedly have filtration, aspiration and deflection as their essential bases are considered in the present paper, and some of the more significant statements supported by the observations are: Fluid normally filters out of the pulmonary capillaries during expiration; during inspiration, much of the fluid is aspirated and deflected back into the pulmonary capillaries after the filtrate has had ample opportunity to give up some carbon dioxide and to take on some oxygen; some of the filtrate leaves with the exhaled air and some of it leaves via lymph channels; a sufficient amount of oxygen can be aspirated into the pulmonary capillaries, when the inflation of the lungs is sudden and strong, to elicit the chemical apnea which is commonly associated with the names Hering and Breuer; it is unnecessary to assume oxygen secretion at the lungs under any conditions.

Observations. A lobe of lung from a dog was placed in an artificial chest appropriately constructed to permit inflation and deflation of the lobe by altering the air pressure in the space surrounding the tissue. A cannula from the bronchus communicated with the room air, and two cannulas from the afferent and efferent pulmonary vessels also protruded from the artificial chest and were generally connected with the central stumps of afferent and efferent vessels in the thigh or in some other part of the body of a second dog. This second dog, which was in ether anesthesia, breathed spontaneously throughout the experiment except when the breathing was temporarily stopped by strongly inflating the transplanted lobe. Coagulation of the blood was guarded against by giving intravenous injections of hirudin to both dogs of a pair before any lobe was transplanted. In order to know the air pressure about the lobe at any time, a glass tube was passed through the wall of the artificial chest and connected with a mercury manometer. The lobe was inflated in all cases by creating a negative air pressure in the artificial chest by means of a suction pump, and deflation of the lobe was accomplished by destroying the negative pressure about the

lobe. The artificial chest was quite successfully employed in working with nine pairs of dogs. In some of the experiments, the artificial chest, with the lobe in it, rested on the platform of a scale so that the weight of the lobe could be determined when desired.

If the artificial chest remained open so that the air pressure in it was the same as that in the room, the lobe soon became firm and heavy because of excess fluid in it. In some instances, almost clear fluid dripped from the end of the bronchial cannula. Some of the lobes appeared to become somewhat edematous—and at least one of them increased appreciably in weight—when these were subjected to a protracted negative air pressure of about fifteen millimeters of mercury; but lobes that were greatly waterlogged at the beginning of the protracted inflation became slowly more normal without becoming entirely so.

If a lobe contained much fluid at the beginning of rhythmic inflation and was inflated rather strongly at the rate of about twenty times per minute, the edematous condition disappeared so rapidly that the tissue was soon almost normal. The rate at which the excess fluid disappeared varied considerably with the individual dogs from which the lobes were taken, with the blood pressures of the living dogs and with the extent and rate of the individual inflations. It did not seem possible to eliminate the excess fluid entirely, for any lobe always weighed a little more after having once been waterlogged. This permanent after-effect of the water-logging could have been due to the fact that no provision was made for the usual drainage of lymph from the lobe. The superficial lymph vessels were observed to be greatly distended with fluid in some cases.

So far the results indicate more or less clearly that fluid filters out of the tortuous capillaries of a deflated lobe, that the blood streams in the linearly extended capillaries of an inflated water-logged lobe deflect the filtrate back into the pulmonary capillaries, that an extracapillary fluid is aspirated into the capillaries during the time that these are undergoing a sudden change from a state of tortuosity to one of relative non-tortuosity and that some of the filtrate normally escapes from a lobe via its air passages and lymph channels. The deflection of the filtrate into the linearly extended capillaries would seem to necessitate especially rapid streams of blood in these capillaries. This is probably the case, for the amount of blood circulating in a unit of time through a transplanted lobe is greater when the lobe is inflated than when it is deflated, as will be shown in a later paper of this series. It is conceivable that at least some of the pulmonary capillaries become virtually Venturi tubes when a lobe is inflated and that deflection of the filtrate takes place through the porous walls at the neck of each Venturi tube. Each of these pores should be considered as a piezometer tube with a length equal to the thickness of the wall of the neck of the Venturi tube.

It was further observed in a number of instances that when a lobe of lung in the artificial chest was very suddenly and strongly inflated, an enormous number of barely perceptible bubbles and many larger ones circulated away in the systemic vein. These bubbles could be seen in the vein almost as well as in the glass cannula connecting the two venous stumps. The spontaneous respirations of some of the dogs, not all, varied in different ways a short time after a transplanted lobe was strongly inflated. In some cases the breathing became exaggerated, but in other instances it became quieter and shallower or even ceased altogether for a short time. The breathing was never immediately augmented and apnea was more regularly elicited by inflating a lobe when the afferent vessel was connected with the central stump and the efferent vessel with the peripheral stump of a severed common carotid artery. Figure 1 is a record of one of the most spectacular instances of apnea caused by inflating a transplanted lobe.

Careful examination of the lobes that had been strongly enough inflated to cause apnea revealed in some instances a certain amount of emphysema but otherwise no abnormal condition that might seem to be associated in any way with the development of the bubbles. Two kinds of emphysema were therefore produced. One of these was interstitial emphysema and the other one was the intracapillary emphysema which was very transient and more physiological than pathological in nature. It was necessary to deflate the lobe in order to learn that an excess of air had entered the interstices, and it was necessary to observe the bubbles circulating away from the lobe in order to learn that an excess of air had entered the capillaries of the lobe. The excess air was evidently aspirated from the alveoli into the interstices and capillaries during the inflation of the lobe, for the air pressure in the artificial chest was at no time greater than the air pressure in the room. Moreover, it was observed that bubbles circulated away from the lobe for a short time while it was inflated.

In another series of experiments, lungs of dogs were suddenly and strongly inflated either by injecting air into the lungs by way of the trachea or by mechanically aspirating air out of the chest. Numerous small bubbles often circulated away from the lungs in these experiments also. The bubbles were especially visible when air was injected through the trachea. A slightly arched glass tube was used to replace a segment of a common carotid artery so that the bubbles could be clearly seen as they circulated to the head. Some of the dogs, not all, ceased breathing during the inflation as well as for a few seconds after the lungs were permitted to deflate. Some of the dogs did not exhibit apnea until the bubbles had had somewhat more than ample opportunity to reach the medulla oblongata. In order to elicit apnea in non-vagotomized dogs, it was in cases not necessary to inflate the lungs so strongly that perceptible bubbles appeared in the carotid tube, but bubbles, and many of them, were required to cause apnea in the same animals after the vagi were cut.

As a biological test for oxygen in the bubbles, pure air, oxygen and carbon dioxid were injected separately and in various combinations into a common carotid artery. Some of the dogs, not all, likewise exhibited temporary apnea after receiving injections of air (fig. 2). It appears quite

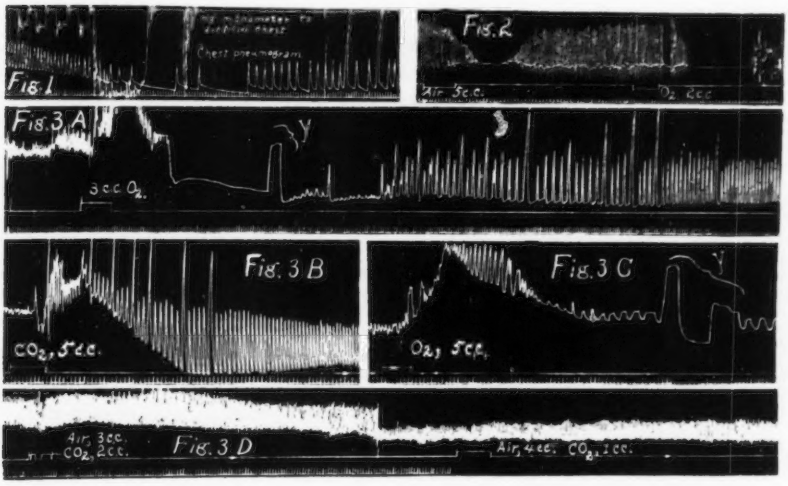


Fig. 1. A 5.5 kilogram dog in ether anesthesia. Apnea caused by inflating a large lobe of lung that was connected with the central and peripheral stumps of the right common carotid artery. The lobe was taken from a 17.2 kilogram dog. The lobe was in an artificial chest and was inflated by creating a negative air pressure about it. The upper tracing shows the air pressure variations in the artificial chest, down stroke meaning inflation of the lobe. The middle tracing is a chest pneumogram from the small dog, up stroke indicating spontaneous inspiration. Lower tracing, time in seconds. The ordinates were transferred with a compass.

Fig. 2. Apnea caused by injecting oxygen and air into the right common carotid artery of a 5.0 kilogram dog in ether anesthesia. The respirations were recorded with a tambour connected to a side arm of the ether bottle. Up stroke, inspiration. The gases were measured with a hypodermic syringe. Time in seconds. Down stroke in signal line, time of injection.

Fig. 3. Probably a 9 kilogram dog (animal not weighed). The heavy end of an unbalanced rod of iron was laid across the sternum. A thread from the light end of the rod passed up over a small pulley and down to a muscle lever. Up stroke, inspiration. Time in seconds. Gases measured by same crude method as for figure 2. Up stroke in signal line, time of injection. Dog in ether anesthesia.

*Y* in A or C of figure 3 was caused by a reaction which had a great resemblance to yawning. A, before any $CO_2$ had been injected. B, fourteen minutes after A. C, six minutes after B. First part of D, about twenty minutes after C. Second part of D, about five minutes after the first part of D. The initial upward trend of the curve in A, B or C was caused by a general bodily reaction which did not invariably occur when a gas was injected.

unnecessary to assume that the apnea was due to some conceivable effect of the emboli *per se*, for pure oxygen injected in the same way caused greater periods of apnea (figs. 2 and 3A) and carbon dioxid alone caused an increase instead of a decrease in the breathing (fig. 3B). Moreover, certain mixtures of oxygen and carbon dioxid or of air and carbon dioxid caused little or no change in the breathing (fig. 3D).

Two special peculiarities of the tracings obtained by injecting oxygen or air several minutes after injecting carbon dioxid are shown in figure 3C. There occurred first an increase in the breathing and then a long period of mild breathing or incomplete apnea, whereas figure 3A (obtained before any carbon dioxid had been injected) shows that a smaller amount of oxygen caused essentially a sustained period of complete apnea. These features of figure 3C might mean that some bubbles of carbon dioxid from the previous injection of this gas lodged and lingered at some point or points in the circulation and were dislodged and driven toward the medulla oblongata by the oxygen bubbles that were later injected, whereupon there was exhibited for a short time an almost pure carbon dioxid effect which was followed by the combined effects of carbon dioxid and oxygen.

The very deep inspirations lettered *Y* (because they had a great resemblance to yawning) in A and C of figure 3 often occurred after considerable periods of either complete apnea or very slow and mild reactions of the chest. Such deep inspirations should serve to eliminate (by aspiration and deflection) the excess filtrate which accumulates in the lungs during such long periods of apnea as that recorded in figure 3A. Liquid should also collect in the lungs during such slow, easy breathing as that preceding the Y-reaction in figure 3C. If this reasoning is correct, the further reasoning is probably correct that the Y-reaction in figure 3C occurred for the same reason and is basically the same as the yawning of a fatigued person a few minutes after he begins to rest. Common observation shows that yawning of human beings and animals is generally preceded by a series of respirations that are unusually shallow and slow, probably so shallow and slow that fluid is filtered out of the pulmonary capillaries more rapidly than it is aspirated and deflected back into them. Animals often interrupt their panting by breathing very deeply in ways that cause one to think at times of sighs and at other times of yawns, yet the reactions may be neither typical yawns nor sighs. Strong and rapid panting of the cat and ferret is sometimes interrupted more or less periodically by tetanic spasms which are essentially very powerful inspirations. There are some observations that indicate that these periodic reactions cannot all be due to oxygen-want alone, to alkalosis alone or even to a combination of these possible factors. Whatever the immediate cause or causes may be, these reactions seem to be safeguards against an animal's drowning in its own plasma, i.e., a filtrate of the blood in the lungs.

Some evidence that the fluid in the lungs is the principal stimulus for at least some periodic and deep inspirations was obtained from a young, vigorous airedale dog. The chest was opened under artificial respiration, the bronchus of the lower left lobe of lung was carefully and securely clamped, ligatures were placed loosely about the artery and vein and the lobe was then deflated by aspirating air out of the bronchus with a large hypodermic syringe. The animal persistently exhibited respiratory reactions, and after about two minutes certain of the abortive respirations became especially strong, somewhat as in the latter part of figure 3A. Upon injecting a few cubic centimeters of a 0.9 per cent salt solution and a small amount of hirudin in the bronchus peripherally to the clamp, the extra strong, periodic respirations became somewhat stronger and more frequent. The afferent and efferent blood vessels were then ligated and the lobe was quickly removed by severing the bronchus and blood vessels peripherally to the clamp and ligatures. This completely eliminated the Cheyne-Stokes nature of the abortive respirations without modifying them in any other way. This periodic breathing was surely quite independent of either acapnia or anoxemia and was in all probability excited by excess fluid in the lobe.

It would be a mistake, however, to assume that an excess of fluid in the lungs is the immediate cause of all forms of Cheyne-Stokes breathing. For instance, it is scarcely conceivable that the excess fluid can be the immediate cause of the periodic breathing of decapitated animals such as the kitten, muskrat, seal (Swindle, 1926) or duck. It probably contributes, however, to periodicity in the breathing of human beings and animals that are subjected to especially low barometric pressures, for, under this condition of diminished external pressure, the relatively high air pressure in the chest cavity should tend to deflate the lungs sufficiently for them to become edematous. Some of the yawning and sighing and also some of the paroxysms consisting of abnormally strong and sudden respiratory reactions exhibited by some humans (not all) and some animals (not all) should tend to eliminate the filtrate that elicits the unusual respirations. Incidentally, the respirations of unusual character might serve in some people and some animals to aspirate and deflect oxygen into the blood in such excess that one who is aware of this excess and who is not aware of the physical factors herein spoken of as aspiration and deflection at the lungs might be inclined to assume, with Bohr (1891), Haldane (1922) and others, that oxygen must be actively secreted into the blood by the lung epithelium. As one may readily learn from Liljestrand's recent survey of the situation relative to the oxygen content of the blood of animals and people subjected to low barometric pressures (1925), the data from different sources are not entirely consistent and are quite contradictory in some respects. Certain discrepancies in these data can be readily accounted

for, however, when the fact is considered that, for some more or less imperfectly unknown reasons, the respiratory mechanisms are much more profundly affected in some people and some animals than in others. Not only are human or animal respiratory cages of such different normal and potential capacities that some discrepancies should be expected, but the state of health of a single individual might give rise to one result rather than to another possible one. My own breathing in the Swiss Alps often contained much stronger and more sudden inspirations than I have been able to observe in myself at even considerably greater altitudes in the Rocky Mountains. This difference should probably be attributed to the facts that I was never in the Alps except immediately after leading the rather sedentary life of a student for several months and that I was never in the Rockies when my physical condition did not seem to be at its best.

## CONCLUSIONS

1. Fluid normally filters out of the tortuous pulmonary capillaries at expiration and much of the filtrate is normally deflected back into the capillaries when they become relatively non-tortuous at inspiration. Some of the filtrate normally leaves the lungs with the exhaled air, some of it normally leaves via the lymph channels and some of it normally leaves via the pulmonary veins.

2. Filtration out of the pulmonary capillaries of carbon dioxid laden plasma and the deflection of the filtrate back into the capillaries after it has had ample opportunity to give up some carbon dioxid and to take on some oxygen are some physical factors which facilitate the process of simple diffusion of gases at the lungs.

3. Unusually deep and sudden inspirations permit extracapillary fluids in the lungs to enter the capillaries by aspiration as well as by deflection.

4. Yawning, sighing and some other deep inspirations are probably elicited by excess filtrate in the lungs and are safeguards against an animal's drowning in its own plasma. Incidentally, so much air may be aspirated from the alveoli into the blood that chemical apnea results or that the oxygen tension in the arterial blood merely increases to a point beyond that which can well be accounted for by considering unaided diffusion of this gas at the lungs.

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# MECHANICAL FACTORS CONTRIBUTING TO THE EXCHANGE OF FLUIDS IN THE BODY

## III. In the Aortic Intercostal Arteries of the Mammal

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The present investigation concerns principally the ejection of blood from the thoracic aorta through the mouths of such piezometer tubes as the left subclavian and the descending aortic intercostal arteries and the return of a part of the ejected blood to the descending thoracic aorta through the piezometer tubes that are commonly known as the ascending aortic intercostal arteries. Figure 1 shows, perhaps better than it is commonly known, that the intercostal arteries are channels of such size that they should offer very little resistance to the flow of blood through them from the descending thoracic aorta to the internal mammary and musculophrenic arteries or vice versa. A fact which is well known, but which is not shown in figure 1, is that some branches of the aortic intercostal arteries anastomose with branches of the inferior vertebral arteries in the spinal canal. Another fact of considerable interest is that the intercostal arteries are associated with one another by numerous large and small anastomoses; it is not impossible, therefore, for blood to circulate from the descending to the ascending aortic intercostal arteries. A photograph of a cleared specimen which shows all of the arterial anastomoses of the intercostal arteries is often an unanalyzable mass of black.

Some of the peculiarities of the blood flow in the channels connecting the internal mammary arteries and the descending thoracic aorta may be gleaned from the circumstances that the ascending aortic stumps may or may not bleed when the channels are severed, that the descending aortic stumps tend in all cases to bleed more profusely than do the ascending ones and that the stumps of the branches from the internal mammary arteries invariably bleed; we might suspect, therefore, that blood sometimes circulates from the internal mammary arteries to the descending thoracic aorta via the ascending aortic intercostal arteries.

In some animals in which there was partial or complete hydrothorax at the time of death, some of the ascending aortic intercostal arteries were so completely occluded or obliterated that it was not possible to inject them.

It would seem from this as if the fluid is normally eliminated from the pleural chambers through the ascending aortic intercostal arteries. Other biological purposes of the centripetal blood streams in the ascending aortic intercostal arteries will be pointed out subsequently.

EXPERIMENTS AND OBSERVATIONS ON DEAD ANIMALS. The trachea and lungs of a doguera baboon were removed, the aorta was severed and

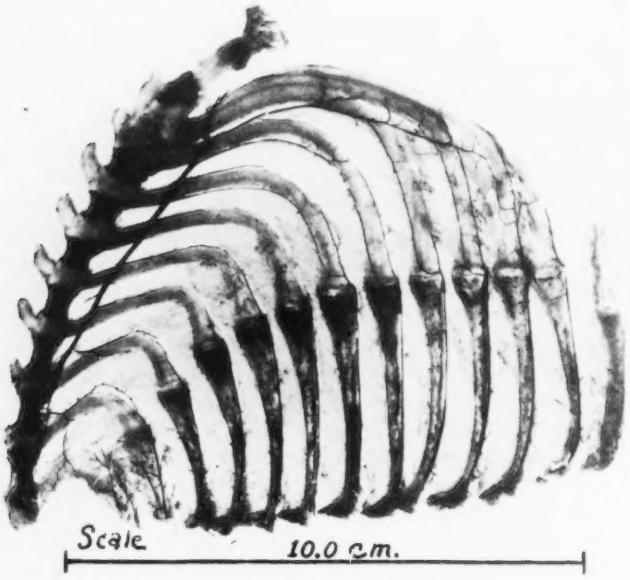


Fig. 1. Left chest wall of a thirty-three day old Bengal tiger. Injected with India ink. No veins injected. Numerous anastomoses not injected. As is also true of all other mammals examined, the intercostal arteries are very distinct arterial highways that associate the internal mammary, musculo-phrenic and a number of other arteries with the descending thoracic aorta. Note the monopolar and bipolar rete mirabile.

cannulated a short distance cephalad to the openings of the most anterior aortic intercostal arteries, the heart was completely removed, the aortic cannula was connected to a water faucet, the abdomen was opened widely, the abdominal aorta was severed a short distance anteriorly to the point of origin of the common iliac arteries and nine hypodermic needles of the same size were inserted into the first nine of the aortic intercostal arteries on the left side. The influence of streams of water in the aorta upon fluid in the aortic intercostal arteries was then determined.

When the rate of flow of water from the faucet was about four meters per second (the amount of water escaping in one second divided by the cross section of the smallest part of the aortic cannula), air bubbles entered the most cephalic artery (from the needle in it) and passed rapidly into the aorta. The number of the bubbles and the rate of movement of them in the more caudal of these arteries diminished progressively until there was stasis in the seventh and eighth and a slightly centrifugal stream in the ninth artery. Ink and other colored fluids, that were dropped into the free ends of the hypodermic needles, likewise flowed at different rates into the aorta from the ascending aortic intercostal arteries. Similar results were obtained from a number of other animals, among them a chacma baboon, a chimpanzee, a kangaroo, a mountain sheep, a skunk and a seal.

In the course of the work with the doguera baboon, it was noticed that the pleural lining of the right chest wall was very dry and crisp from about the second to the sixth rib and that the corresponding area on the left side was quite moist. Water had been flowing through the aorta for about two hours when the observation was first made. Both sides were then repeatedly moistened, and the right side always became noticeably drier than the left one in about fifteen minutes. Since the most important difference between the two sides appeared to be that the right internal mammary and musculo-phrenic arteries were cut away in the process of removing a part of the right chest wall, the left musculo-phrenic artery was now ligated near its origin. This was done to keep water from circulating from the aorta into the ascending aortic intercostal arteries via the descending aortic intercostal arteries, the musculo-phrenic artery and the internal mammary artery. The rate of drying of the two sides was then about the same, and the left side dried faster than the right one after the aortic intercostal arteries on the right side were ligated at and very near the aorta. These observations suggest that fluid normally enters the pleural chambers of a living animal from the descending aortic intercostal arteries and that it is normally eliminated from these chambers through the ascending aortic intercostal arteries. The elimination might be especially rapid when the arterial blood pressure is very low and the flow of blood in the aorta is abnormally rapid.

In the dead animal, the fluid passed from the descending to the ascending aortic intercostal arteries principally through the musculo-phrenic and internal mammary arteries. This is an unusual route that can in all probability be followed in the dead animal only. In the living animal, whole blood can pass from the descending to the ascending aortic intercostal arteries through numerous small arteries that unite the aortic intercostal arteries with one another.

Upon gradually diminishing the rate of flow of water in the aorta, the centripetal streams gave way to centrifugal ones in an orderly sequence,

the last to become centrifugal being those in the more anterior aortic branches. The axial and peripheral streams in the aorta were probably now so much alike that deflection into the aorta was not possible.

Upon gradually increasing the rate of flow from the faucet beyond that of about four meters per second, the centripetal streams likewise gave way to centrifugal ones. The aorta soon collapsed to a certain extent. The lateral walls seemed to lack the necessary rigidity to resist being deflected toward the axial stream in the aorta. These walls accordingly fell in upon the axial stream and, in so doing, greatly lessened the difference between the axial and peripheral streams that would otherwise have existed at this time and which is essential for good deflection of the fluid portions of the aortic walls, as in the mouths of the ascending aortic intercostal arteries. The streams in all of the aortic branches were now centrifugal. Upon increasing the rate of flow from the faucet still more, the aorta became distended and the streams in all of the aortic intercostal arteries became strongly centrifugal.

In working with a second doguera baboon, the right chest wall was almost completely removed by cutting the costal cartilages close to the sternum, by cutting just anteriorly to the diaphragm and by cutting each rib close to the spinal column. The aortic branches to the right side were ligated. The left internal mammary artery was cannulated near its origin from the subclavian artery and the cannula was connected to an upright burette. Otherwise, this baboon was prepared in the same way as the first one.

A colored solution of sodium citrate flowed from the burette into the aorta through the intercostal arteries. The rate of flow varied with the hydrostatic pressure in the burette and accordingly in the internal mammary artery. The rate of escape of the solution from the burette could be influenced by permitting water from the faucet to flow through the aorta at different rates. When the hydrostatic pressure in the burette amounted to about thirty millimeters—the burette being filled up to this level about as fast as the solution escaped—the rates of flow in the aorta necessary to increase the rate of escape of the solution from the burette ranged from about two (the minimal flow) to about six and one-half meters (the maximal flow) per second. The rate of flow (the optimal flow) in the aorta which permitted the most rapid escape of the solution from the burette was about five meters per second.

Several tests showed that only a few millimeters change in the hydrostatic pressure had a considerable influence upon the range of possibilities of either augmenting or creating centripetal streams in the ascending aortic intercostal arteries of the doguera baboon and some other animals. This was especially true of a buffalo calf in wlich all of the aortic intercostal arteries were ascending. The influence of the hydrostatic pressure was

great enough in the different animals that we may safely assume an especially strong tendency of blood in the living mammal to flow from the arch of the aorta into the descending thoracic aorta via the subclavian, internal mammary and ascending aortic intercostal arteries. If this circus movement of the blood should occur in the living mammal, the intercostal arteries would not be deprived of an adequate supply of arterial blood and fluid would be drained from the pleural chambers during the time. In case of little or no deflection into the aorta, complete stasis would not exist in the ascending aortic intercostal arteries because the blood in these arteries would then enter the intercostal veins in greater amounts than usual; but this condition of relative stasis would not favor elimination of fluid from the pleural chambers.

Hydrostatic pressures created in an inferior vertebral artery of a mountain sheep yielded essentially the same results as were obtained by establishing hydrostatic pressures in an internal mammary artery of the second doguera baboon. In both animals the rate of flow of a solution from a burette into the aorta via an internal mammary artery or an inferior vertebral artery was greatly altered by permitting water to flow at different rates through the aorta. If the circus movement of the blood via the inferior vertebral artery ever occurs in the living mammal, a portion of the spinal cord and its meninges would not be deprived of an adequate supply of arterial blood during the time and spinal fluid might be drained from the spinal canal into the aorta.

It was not possible to create centripetal streams in any of the aortic intercostal arteries of some animals, especially dogs, by merely forcing water through the aorta. It was necessary in such cases to establish a small hydrostatic pressure either in the internal mammary or the inferior vertebral artery. The important differences between these animals and others seemed to lie in the shape of the mouths of the aortic intercostal arteries.

Experiments and observations on living animals. Segments of two ribs were removed in each of a considerable number of dogs to make a circular opening into a pleural chamber. The mouth of a glass funnel was placed over the opening and the small end of the funnel was connected to a suction apparatus. The lungs could then be inflated as desired by diminishing the air pressure in the chest. The usual breathing of the animals was not seriously disturbed. In almost every case in which there was no spontaneous bleeding from the aortic stumps of the aortic intercostal arteries and in which the failure of the stumps to bleed was not due to clotted blood in them, blood could be caused to flow freely from the stumps by creating an appropriate negative air pressure in the funnel and chest. The negative pressure required for this varied considerably for some of the dogs and also for any one dog at different times. The force artificially

applied to aspirate the blood out of the aorta when there was otherwise no bleeding must have more than compensated for a similar force acting in the opposite direction to cause blood or other fluid to flow into the aorta instead. The arterial blood pressure and the rate of flow of blood in the aorta were evidently such that fluid was deflected from the aortic stumps into the aorta.

Deflection did not occur and the stumps bled when the femoral arteries were compressed. Intravenous injections of adrenalin also caused the stumps to bleed as long as the arterial pressure was quite high. Inhalation of amyl nitrite caused any spontaneous bleeding of stumps of ascending aortic intercostal arteries to cease, and the deflection was at times quite strong, for it was often not possible to overcome the deflection by sucking air out of the chest. In some of the attempts to counteract the deflection at the aorta, the lungs were so greatly inflated that lung tissue filled the funnel sufficiently to occlude the opening to the suction apparatus.

A small glass cannula that had been in a ten per cent solution of sodium citrate was inserted into the aortic stump of the fourth aortic intercostal artery of a very large dog. After all of the other cut arteries were ligated, the glass funnel was placed over the opening in the chest wall and a long aspirating needle was inserted through the wall of the chest and into the free end of the cannula. The normal air pressure was created in the chest and suction was applied to the funnel whenever necessary to cause a selected edge of a lobe of lung to reach a certain point at the edge of the opening at each inspiration. A one per cent solution of sodium citrate was dropped as rapidly as desired into the large end of the apsirating needle to prevent coagulation of the blood in the cannula. Incidentally, the citrate solution served to prevent or reduce aspiration of air into the aorta at expiration and to wash the cannula sufficiently that the level of the fluid in it could be seen. The arterial blood pressure was recorded from a common carotid artery. The crests and troughs of the respiratory waves in the aterial blood pressure were considered as the systolic and diastolic blood pressures. The length of the cannula from the ligature to the free end was forty millimeters.

At each inspiration (systolic pressure 143 mm. Hg) the solution passed rapidly through the needle and into the cannula and blood-colored fluid escaped from the top of the cannula at only some of the inspirations although the breathing appeared to be quite regular. At each expiration (diastolic pressure 104 mm. Hg) the fluid in the cannula sometimes disappeared entirely, but the solution was ordinarily not forced back through the needle sufficiently for an appreciable amount of air to escape from the chest. Upon compressing both femoral arteries, while citrate was not being dropped into the needle, blood ran over the top of the cannula at each inspiration (systolic pressure 152 mm. Hg), and at expiration (diastolic

pressure 114 mm. Hg) the fluid often sank nine or ten millimeters in the cannula. An intravenous injection of about four cubic millimeters of a 1:1000 solution of adrenalin caused blood to run over the top of the cannula at expiration as well as at inspiration. In order to prevent excessive loss of blood through the cannula, the animal was allowed to inhale amyl nitrite while the blood pressure was still quite high. After the systolic and diastolic pressures were reduced to 117 and 95 mm. Hg respectively and while the citrate was dropped as rapidly into the needle as it escaped into the cannula, the cannula never became more than about half full.

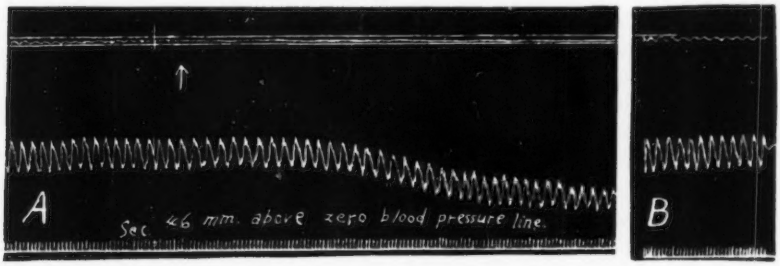


Fig. 2. Effects of amyl nitrite inhalation on the blood pressure in the aortic stump of the fourth aortic intercostal artery of a large dog. The upper tracing between the two horizontal lines is the mercury manometer tracing from the stump. The upper horizontal line is the extension of the zero blood pressure line on a part of the paper not shown here. The lower horizontal line shows the lowest level to which the blood pressure in the stump fell during the inhalation of amyl nitrite. The first tracing below the arrow was obtained with a mercury manometer from the right common carotid artery. The base of the time line in A is 46 mm. above the zero blood pressure line for the carotid tracing. B was secured about 35 minutes after the dog ceased to inhale amyl nitrite. The time tracing is lower in B than in A because the level of the chronoscope was unintentionally altered while winding it a short time before B was recorded. The arrow indicates the beginning of inhalation of amyl nitrite.

There was not a great difference in the level of the fluid in the cannula at inspiration and expiration. The difference was never greater than about five millimeters, and at least some of this difference was evidently due to the different rates of entrance of the citrate solution from the needle at inspiration and expiration. When the solution was not dropped into the needle for a short time, all of the liquid in the cannula vanished into the artery.

In order to secure some graphic records of the pressure variations in the cannulated stump, the aspirating needle was removed and the cannula was connected to a mercury manometer. The rubber tubing used to make the connection had such thick walls that it was evidently not affected by the air pressure changes in the chest at inspiration and expiration.

This tube passed through an opening of appropriate size in the chest wall. In order to determine the zero pressure in the system, a stop-cock was connected to the side arm of a T-tube inserted in the rubber tube outside the chest and the artery was compressed with a blunt edge near the cannula. Figure 2 was obtained before, during and after inhalation of amyl nitrite. The results are not very imposing, but they confirm some of the more important observations already reported in this division of the paper. The tracing of primary interest is in the upper part of the figure and is between two horizontal lines, the upper line being the zero blood pressure level and the lower one being the lowest level reached by the blood pressure in the stump during the inhalation of amyl nitrite. The only time that even the crests of the respiratory waves in the tracing reached the zero blood pressure level was some time after the recovery from amyl nitrite (fig. 2B). The blood pressure in the stump was at no time above the zero pressure level.

The experiments on the living animals were performed under unfavorable conditions in so far as the blood pressure in the internal mammary artery was not permitted to augment the centripetal streams of blood in the ascending aortic intercostal arteries. The centripetal streams that were determined would undoubtedly have been much greater if the arterial channels connecting the internal mammary artery and the aorta had not been cut. It is reasonable, therefore, to assume that blood normally flows into the aorta from the ascending aortic intercostal arteries.

## SUMMARY AND CONCLUSIONS

1. The results indicate that under normal conditions of circulation, blood is both pulled and pushed into the aorta through the ascending aortic intercostal arteries. This blood comes mainly from the internal mammary and inferior vertebral arteries, but some of it probably comes from the descending aortic intercostal arteries through numerous small arterial anastomoses in the intercostal muscles.

2. Under certain conditions of circulation, as when amyl nitrite is inhaled, fluid is deflected into the descending thoracic aorta from the stumps of the severed ascending aortic intercostal arteries. The deflection is only stronger when the fluid is urged toward the aorta by the fluid pressure in the internal mammary, inferior vertebral and descending aortic intercostal arteries.

3. Whenever the pulled and pushed blood rushes quite rapidly through the arterial highways connecting the internal mammary arteries with the descending thoracic aorta, fluids are deflected into these channels from the intercostal muscles, the pleurae, the pleural chambers and a part of the neural canal, and the tissues in the vicinity of the heterocladic channels are not deprived of an adequate supply of oxygen during the time.

4. The blood arriving at the aorta from the aortic intercostal arteries is necessarily more acid than that coming from the left heart. In view of the well-known fact that an acid medium facilitates the dissociation of the oxyhemoglobin compound, the acidified fluid that enters the thoracic aorta should enable the blood to give up its oxygen quite readily when it reaches the capillaries at various parts of the body.

# MECHANICAL FACTORS CONTRIBUTING TO THE EXCHANGE OF FLUIDS IN THE BODY

## IV. In the Lungs and the Ascending Branches of the Descending Thoracic Aorta of the Bird

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The present paper concerns primarily the following situations relative to the lungs and their system of drainage canals in the bird: The pulmonary capillaries have extremely thin walls and are quite tortuous and liquid constantly filters out of them, as occurs at expiration only in the mammalian lung; the bird lung does not inflate at inspiration and the filtrate accordingly has not the opportunity, as it has in the mammalian lung, to be aspirated and deflected back into the pulmonary capillaries at inspiration; the ascending branches of the descending thoracic aorta turn much more sharply forward in the bird (figs. 1 and 2) than in the mammal, and they constitute a drainage system of such efficiency in the bird that it is not necessary for the filtrate to return to the circulation by any other route; inhalation of enough dust to cause dust fever in a mammal causes essentially a fall in the temperature of a bird (figs. 5 and 6); a bird's breath cannot be seen on a cold day unless an especially large volume of air is exhaled, as when the bird cries, or unless the drainage system for the thoracic region is sufficiently blocked that the usual amount of filtrate is not deflected from the lungs into the aorta.

Some points of secondary importance in connection with the thoracic region of the bird are: The respiratory surface is probably smaller than that of even the collapsed lung (at expiration) of a mammal of the same weight; it has no alveoli; air probably passes through all parts of it at each respiration; a bird (duck) ordinarily expels more air at each expiration than does a mammal (rabbit) of the same weight; the greater part of the diaphragm lies snugly upon and is rather firmly attached to the pleural covering of the ventral surface of the lung (fig. 3); the muscles of the diaphragm (fig. 3) do not react visibly during normal or even dyspneic breathing; the right ventricle of the heart is considerably less hypertrophied than in the mammal; the greater portion of the respiratory parenchyma of an exposed lung can be dissected away from the pulmonary

artery and vein and their larger branches without causing a great loss of blood; if bleeding occurs from some of the smaller pulmonary blood vessels, it may be quickly stopped by rubbing the finger lightly over the cut surfaces, but this does not prevent a profuse flow of clear fluid from the wound; hemorrhages occur rather frequently in the lungs (especially in the left one) of birds, but the process of repair is rapid and certain unless the point of hemorrhage is in one of the larger branches of the pulmonary artery or vein; the temperature of the bird is normally higher than that of the mammal; the ascending branches of the descending thoracic aorta connect the lower third of this segment of the aorta with the inferior vertebral, the intercostal, the musculo-phrenic and the internal thoracic arteries; some of the ascending branches of the descending thoracic aorta turn directly forward and lie snugly against the aorta for considerable distances (figs. 1 and 2); it is not uncommon, especially in old or diseased birds, for the greater number or even all of the ascending branches of the descending thoracic aorta to become obliterated due to the mycelium or spores of a fungus, to a yellowish deposit from the lungs or, seemingly, to mere deflection due to the axial blood stream in the aorta; the ascending aortic branches that turn forward most sharply are ordinarily the first to become obliterated; the order of obliteration is generally the right ductus arteriosus, the most anterior of the aortic branches that connects with the vertebral artery of the left side, the left ductus arteriosus, and thereafter the obliteration seems to occur in an irregular order.

EFFECTS OF SPONTANEOUS BLOCKING OF SOME OR ALL OF THE ASCENDING BRANCHES OF THE DESCENDING THORACIC AORTA. A penguin became cold to the touch (the temperature was not taken); it shivered almost constantly, and quite violently on cool days; moisture condensed about its nostrils on cool days when the breathing was normal or about normal, and icicles formed on the short feathers at the base of the beak on very cold days; the bird frequently yawned and often gasped as if it were drowning; upon turning the bird upside down and pulling the trachea in a certain way, a clear, slimy liquid streamed from the mouth, and the streaming did not cease when the esophagus was compressed with the fingers; upon shaking the bird in certain ways, one could hear liquid rattle in the body, presumably in the air sacs. The bird stayed away from a pool of water in its pen. It was not observed to drink, but it usually ate quite well. The facts of primary interest revealed upon examining the bird after death are: a rank growth of a fungus (Aspergillus malignus) on all of the walls of the air sacs; all walls of the air sacs very much thickened, very rigid and extremely vascular, the configurations of the blood vessels in the walls being quite unlike those in a normal penguin; a pulmonary hemorrhage from a rupture of the pulmonary artery immediately underneath the respiratory parenchyma and into the vestibule of the principal bronchus of the left lung;

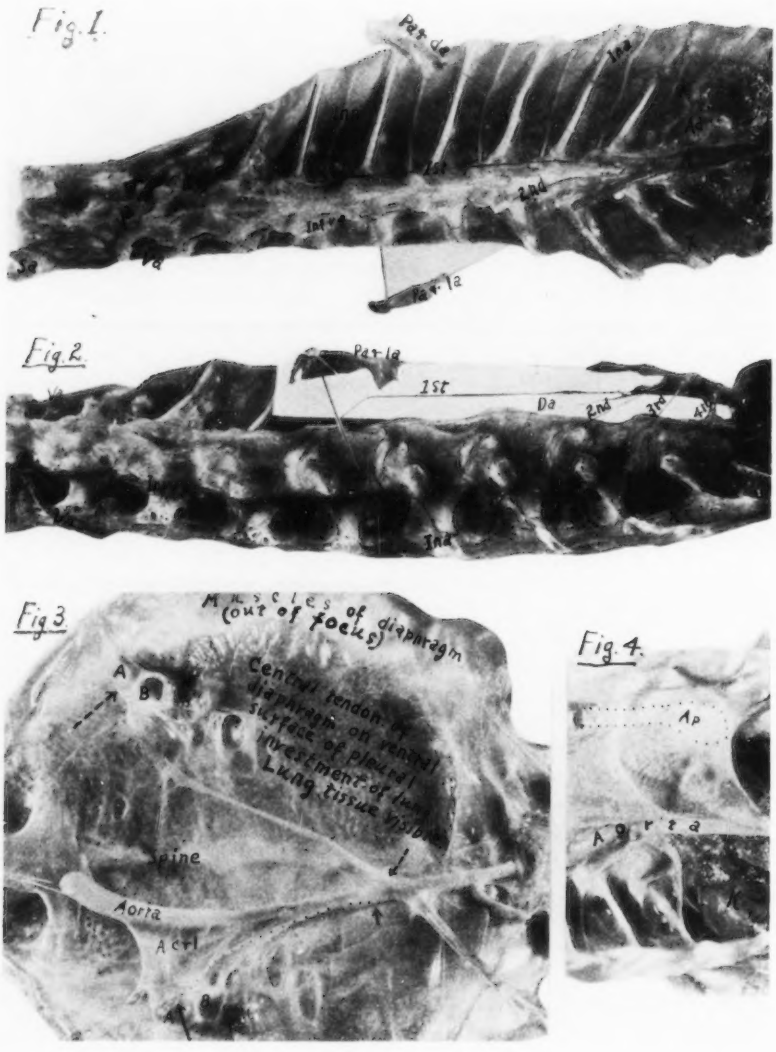


Fig. 1. Thoracic region of a domesticated goose showing the more outstanding parts of the dorsal and ventral drainage systems of the lungs. Ventral view. Lungs removed. The arteries and the left ductus arteriosus injected with India ink by placing the fluid under pressure in the descending thoracic aorta. Cephalic portion of aorta cut away in attempting to show clearly the points of origin of some of the large ascending aortic branches. *Sa*, portion of left subclavian artery thrown for-

ward and over on to the right side. *Va*, vertebral artery. *Inf va*, right inferior vertebral artery. *In n*, intercostal nerve. *Pa* and *da*, a part of the left pulmonary artery, from near the lung, and the ductus arteriosus. *Pa* and *la*, a part of the right pulmonary artery, from near the lung, and the ligamentum arteriosum. *1st*, the most anterior ascending artery; it communicates with the left vertebral (inferior) artery at the second intercostal space. *2nd*, one of the ascending branches that communicates with the right inferior vertebral artery at the fifth intercostal space. The other ascending branches that communicate with the inferior vertebral arteries are not shown in this photograph. *A*, caudal portion of descending thoracic aorta. The aorta is opened from the ventral side. *X*, channels passing to or from the dorsal aspect of the adrenal bodies and connected with the inferior vertebral arteries at the sixth and seventh intercostal spaces. *Ad*, adrenal bodies, the right one lifted out of its normal location and turned over to show more of *X*. *K*, cephalic portion of left kidney. About two-thirds of natural size.

Fig. 2. Same specimen as for figure 1, but is photographed from right side to show especially the relationships of the intercostal arteries and the inferior vertebral artery of the right side to the ascending arteries of the lower thoracic aorta. The segment of aorta is pulled somewhat forward and away from the spine to show the points of entrance of the ascending arteries into the aorta. The aorta is turned to show its dorsal, external surface. The second and third ascending arteries to the left vertebral artery were torn loose from the aorta. The 1st, 2nd, 3rd and 4th are decidedly ascending arteries. *Da*, ductus arteriosus of left side. The other letters mean the same as in figure 1.

Fig. 3. Ventral view of thoracic region of a very old merganser, showing aortic drainage system of ventral surface of lungs. The left ductus arteriosus and its capillaries were mildly injected (with a hypodermic syringe) with milk and immediately afterwards with acetic acid to coagulate the milk and thereby prevent its rapid disappearance. Point of injection near the junction of the duct with the aorta, at the short arrow with the broken shaft. The milk did not flow entirely to the junction of the duct and the left pulmonary artery, at the arrow with the long, broken shaft. The corresponding structure on the right side was already a ligamentum arteriosum and could not be injected. Its junctions with the right pulmonary artery and the aorta are indicated by the solid arrows with long and short shafts respectively. The course of the tendon is indicated by the row of small dots between the solid arrows. *A*, stumps of left and right pulmonary arteries. *B*, stumps of left and right bronchi. *V*, stumps of left and right pulmonary veins. *Ac* and *l*, aortic capillaries and ligaments after first some milk and them some acetic acid was injected into the right lung, immediately under the pleura and a few millimeters anteriorly to the stump of the right pulmonary artery. About two-thirds of natural size.

Fig. 4. Ventral view of caudal portion of thoracic region of a caracara eagle showing a special air pocket, *Ap*, between the tendon of the diaphragm and the pleural covering of the ventral surface of the left lung. First milk and then acetic acid was injected into the pocket. The milk was not under pressure in the pocket, but it escaped quite rapidly toward the aorta by passing especially in the anterior wall of the anterior abdominal air sac. The injection of the milk preceded the injection of acetic acid by only three or four seconds. *Ant ab*, cephalic end of the anterior abdominal air sac. *K*, cephalic end of kidney. Right lung removed. Such an air pocket was on the ventral surface of the right lung also. About two-thirds of natural size.

the principal bronchus and its various branches were completely filled with clotted blood; the ductus arteriosus on each side completely obliterated, probably long before the mycotic infection occurred; all of the other ascending branches of the descending thoracic aorta completely blocked by the mycelium, the microspores and the macrospores of the fungus.

A great blue heron (apparently a very old bird) shivered almost constantly on cold and even relatively warm days, and its breath was quite visible on cold days. The bird yawned and gasped at rather frequent intervals. The essential findings after the death of the bird were: much clear fluid in the air sacs of the right side; much blood-colored fluid in the air sacs of the left side; pulmonary hemorrhage in the left lung, but the exact point of the hemorrhage could not be located; the principal bronchus and its various branches completely filled with clotted blood; extreme anthracosis; deposit of yellow, hard substance in lower thoracic aorta, especially about the mouths of the ascending arteries; ductus arteriosus on each side obliterated; all of the other ascending branches of the descending thoracic aorta so completely obliterated that they could not be injected by putting fluid under pressure in the thoracic aorta; a colored fluid could be forced from the inferior vertebral artery into the internal thoracic artery and vice versa via the intercostal arteries, but the fluid could not be forced into the aorta through the ascending aortic branches.

A twenty-one year old ostrich shivered almost constantly on cool and even agreeably warm days for about three weeks before it died. The breath could be seen at times when the breathing seemed to be normal or about normal, and the bird yawned and gasped at frequent intervals. The chief findings after the death of the bird were: a slimy, slightly blood colored fluid escaped from the mouth when the bird was lifted by the feet, and the fluid continued to escape after the esophagus was securely compressed; marked anthracosis; considerable blood-colored fluid and foam in the air sacs; hemorrhage near the point of origin of one of the large branches of the right pulmonary artery; each ductus arteriosus obliterated; all of the other ascending branches of the descending thoracic aorta also completely obliterated; lower third of descending thoracic aorta quite rigid due to a yellow, hard deposit; the same kind of deposit on the walls of the air sacs; several remnants of hemorrhages in both lungs but especially in the left one.

A squab of about three weeks was brought to the laboratory from a nest on the floor of an extremely dusty cote. A large number of old pigeons, that roosted and nested in this cote, kept dust in the air much of the time. The squab's cloacal temperature was only 96.8 degrees Fahrenheit. The bird shivered almost constantly. A clear fluid streamed from the mouth when the bird was lifted by the feet and its head was pulled down. Yawning was frequent and the squab often gasped as if it were drowning,

but it did not die until late in the afternoon of the second day after it was brought to the laboratory. A large amount of clear fluid was found in its air sacs. A large amount of dust was in the walls (including the central tendon of the diaphragm) of the air sacs, the entire pleural investment of the lungs and in the respiratory parenchyma, yet all of the ascending branches (including the ductus arteriosus on each side) of the descending thoracic aorta, the inferior vertebral arteries, the musculophrenic arteries and the internal thoracic arteries were successfully injected by placing the fluid under pressure in about the caudal half of the thoracic aorta. The dust had evidently occluded only the capillary branches of the system of drainage canals that leads to the aorta. There was no pulmonary hemorrhage.

There was considerable clear fluid in the lungs and air sacs of various other birds in which there was marked anthracosis, but no recent pulmonary hemorrhage, and in which both arterial ducts and at least two of the other ascending branches of the descending thoracic aorta were unmistakably obliterated. Yawning, gasping and shivering were exhibited by some of these birds that were observed before death. These birds, like the penguin, the heron, the ostrich and the squab, evidently drowned in the fluid from the lungs, for all post-mortem findings that did not pertain to the drainage system of the lungs were negative. It was probably the excess fluid in the lungs that excited the yawning and gasping. The shivering and evident subnormal temperatures of so many of the birds were probably due to the circumstance that the drainage system of the lungs no longer functioned in the normal way and accordingly no longer served to conserve a considerable amount of water and incidentally a considerable amount of heat. Brandes (1924) observed that one cannot see the breath of a bird in cold weather unless an exceptionally large volume of air is expelled, as when the bird cries. This is generally true (according to my observations) of normal birds but not of some in which the drainage system of the lungs is partly or completely blocked.

The cloacal temperatures of eleven squabs that were taken singly and in pairs from different nests ranged from 109.4 to 96.8 degrees Fahrenheit. (The one pigeon with the extremely low temperature of 96.8 degrees was considered in an earlier paragraph.) All of the squabs were about three weeks old when selected and brought (by me) to the laboratory. Sex differences with regard to body temperature could not be established. Four of the squabs were taken at the same time from two nests that were about 160 to 100 centimeters above a hay-covered floor of a barn. There was probably very little opportunity for these four squabs to inhale dust. The temperature of each of the two birds from the higher nest was 109.4 and the temperature of each of the birds from the lower nest was 109.2 degrees. One squab taken from the lower nest at a later date had a body

(cloacal) temperature of 109.1 degrees. The cloacal temperatures of two squabs taken from a nest in a small box about forty centimeters above the floor of a very dusty cote were 108.3 and 108.2 degrees, and each of three squabs taken from nests on the floor of the same dusty cote had a cloacal temperature of 106.6 degrees. The one squab with a cloacal temperature of 96.8 degrees was taken from the dustiest cote I have ever seen. The four birds with cloacal temperatures of 109.4 and 109.2 degrees were used in an experiment that will be reported subsequently (fig. 6).

EFFECTS OF PURPOSELY BLOCKING THE DRAINAGE SYSTEM OF THE LUNGS. The marked anthracosis of so many of the birds that shivered, yawned, gasped and even drowned in the fluid from their lungs suggested an experiment, the results of which are presented as curves in figures 5 and 6. A domestic goose, for instance, was placed in a box provided with a glass cover so that the bird could be observed while charcoal dust was blown into the box through a small opening in one end. Special openings in the sides of the box could be regulated to prevent respiratory distress of the bird. The capacity of the box was 25,135 cc. The dusting lasted for about three hours each day. The dust was blown into the box by squeezing a special pressure bulb a few times at intervals of about five to ten minutes. The temperature was taken with a one-minute clinical thermometer at about nine o'clock each morning immediately before the bird was put into the box and when it was not in a state of excitement. At each determination, the thermometer remained in the cloaca for four minutes. With the exception of the old female pigeon (fig. 6), the same was done with all the other experimental birds. While the old female pigeon was being dusted the cloacal temperature was taken two and three times each day, and this bird remained in the box day and night.

The birds did not show distress due to the dusting, i.e., when the dust was blown into the box. Two half-grown rabbits likewise did not appear to be disturbed by a three-hour period of dusting, but they later developed fever (evidently dust fever) and died.

One of the important features of the curves in figures 5 and 6 is the initial difference in temperature of birds of a kind, as the geese or the pigeons. These differences depend somewhat upon the mere age of the birds, as shown by the male control in figure 6, and not upon the diet, as shown by the old female pigeon used as a control in figure 6. All of the birds were given the same kind of food (oats) while they were in the laboratory. The four squabs (fig. 6) were taken from nests located at such places that the birds had little opportunity, if any, to inhale dust. Other squabs secured from nests in dusty places had lower temperatures. The two geese were nine or ten months of age when brought to the laboratory and they were secured from the same farm, but their habits were sufficiently different that they had unequal opportunities to inhale dust.

The male goose roamed the fields during the day, apparently to avoid associating with the other geese on the farm. The other geese, of which the female goose (figure 5) was one, sometimes went into the fields, but they were often in dustier places in and near the barn. It seems, therefore, as if the initial differences in temperature of birds of the same kind are due primarily or entirely to differences in the amount of dust inhaled.

The chief feature of each of the curves (figs. 5 and 6) is the fall due to the dusting. If the drainage system of the lungs is partially blocked, as it must have been by the inhaled dust, the filtrate from the pulmonary capillaries does not reach the aorta in as large amounts as usual and much of it escapes as vapor with the exhaled air. Incidentally, considerable heat also escapes with the exhaled air and the temperature of the body accordingly becomes subnormal.

Perhaps the best way of dealing with some of the marked fluctuations in the curves is to assume that the increase in temperature of the female goose during the dusting, of the male goose on the fourth day after the dusting or of three of the squabs during the dusting indicates dust fever comparable to and subject to the same explanation as dust fever in the mammal. One should then conclude that the duck had intermittent fever during the greater part of the time that it was dusted and for a number of days after the dusting was discontinued.

A possible objection to assuming dust fever in any of the birds is that one of the squabs did not seem to exhibit it. However, there is one important fact which should not be overlooked when attempting to interpret these temperature curves. This fact is that, with one exception, the temperature of any bird was taken only once each day. This means that many interesting features of the curves that could have been appreciated by taking the temperature more frequently, as hourly instead of daily, were lost. The one exception mentioned pertains to the latter part of the curve from the old female pigeon that was used as a control for many days (fig. 6). This bird was finally placed in the box and allowed to remain there day and night, and it was dusted throughout the day from about nine to five o'clock. An effort was made to take its cloacal temperature at nine, one and five o'clock. These temperatures are plotted as a heavy line. This curve shows essentially an initial fall, a rise and a later fall in the temperature. The dotted line, however, which was plotted by considering only the temperatures taken about nine o'clock each morning, shows only an initial rise and a subsequent fall in the temperature; this curve is very much like some dust curves obtained from two rabbits but not shown here.

Probably the most interesting of the situations indicated in figures 5 and 6 are the pulmonary hemorrhages in the male goose and the old female pigeon. As was pointed out earlier in this paper, pulmonary hemor-

rhages are rather frequent occurrences, especially in the left lung, but they ordinarily do not prove fatal unless they occur in the pulmonary artery or one of its large branches. The hemorrhage in the goose was from the pulmonary artery immediately underneath the pleural covering of the

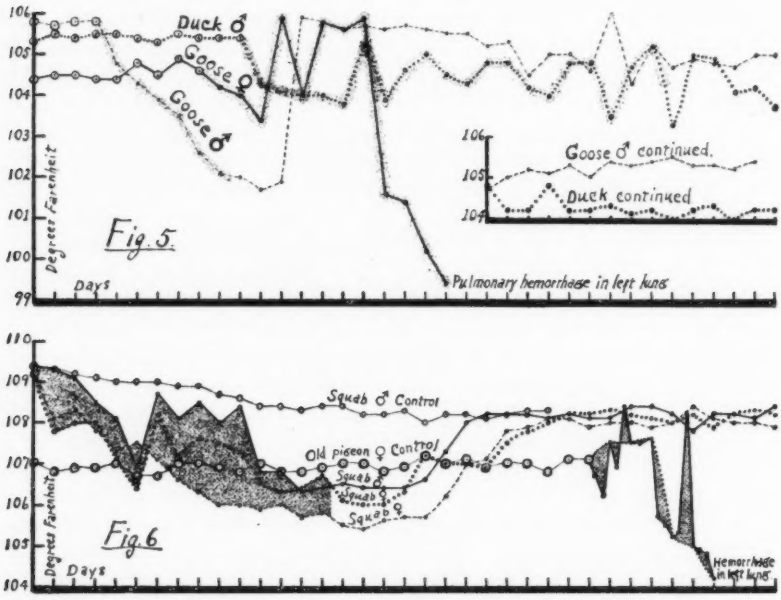


Fig. 5. Effects of dusting two domestic geese and a domesticated muscovy duck. The birds were placed in a box at separate times, and charcoal dust (from oak wood) was blown into the box through a small opening in one end. The dusting was done at about 5 to 10 minutes intervals for about three hours each day. The temperature of each bird was taken, by cloaca, at about 9 o'clock, ordinarily just before the bird was placed in the box to be dusted. The dots in the circles show the temperatures taken before the birds inhaled any of the dust. The stippling about each of the curves indicates the period (the number of days) of dusting.

Fig. 6. Effects of dusting three squabs and one old pigeon. The three squabs were placed in the box together and dusted. The old pigeon was used as a control for many days and was then kept in the box day and night and was dusted from about 9 to 5 o'clock each day. Its temperature was generally taken three times daily, at about 9, 1 and 5 o'clock. Otherwise, the method was the same as described in connection with figure 5. The first stippled area shows the period (the number of days) the three squabs were dusted. The second stippled area shows the number of days the old pigeon was dusted. One male squab was used entirely as a control. The dotted line in the curve from the old pigeon is a record of the temperatures taken each morning, and the heavy line is a record of the temperatures taken two or three times daily.

ventral surface of the lung. The hemorrhage in the pigeon was from one of the large branches about four millimeters from its point of origin from the trunk of the pulmonary artery. The bird lung becomes markedly turgid when it is soaked in plasma. Perhaps due to this turgidity the extremely thin-walled capillaries become occluded in such large numbers that the blood pressure increases sufficiently in the pulmonary artery to rupture this artery or some of its branches. It is not clear why hemorrhages should occur the more frequently in the left lung unless their occurrence is in some way associated with the fact that a considerable portion of the thoracic aorta lies on the ventral surface of the right lung; this relationship might mean that the drainage of the right lung is somewhat better than that of the left one.

The two geese and the duck shivered, even on warm days, when the body temperature was quite low. The shivering of the duck was at times quite violent. Also, when the temperature of the duck or either of the geese was quite low, moisture condensed rapidly on the inner surface of the glass lid of the box and the breath of any of these birds could be seen under such conditions of external temperature and humidity that the breath of the human was not visible as long as his respirations were normal. Upon shaking the body of any of these birds in certain ways, one could hear water rattle, presumably in the air sacs. For two days before the female goose died, it was often observed to gasp, and a clear fluid could be poured out of the mouth by placing the head down and pulling the trachea somewhat strongly. Fluid that was poured out of the mouth after the bird died was distinctly blood colored. Much fluid was found in the air sacs. This was almost clear on the right and quite bloody on the left side, and the same was true of the pigeon that likewise died of a hemorrhage in the left lung. The pigeon gasped quite frequently for at least two hours before it died. Aside from marked anthracosis, the only detectable effects of dusting the three squabs were the changes in body temperature recorded in figure 5.

Special observations on the ascending branches of the descending thoracic aorta. The ductus arteriosus on the left side (fig. 1) may remain completely open for at least three years, as was true of one three year old swan, and for several years thereafter it may be only partially closed, as was true of a very old merganser (fig. 3) and some other old birds. It closes first at or very near the pulmonary artery, as is also true of the right ductus arteriosus of the bird and of the single ductus arteriosus of the mammal. The left duct of the bird is by no means a simple channel. Some of the more fortunate injections of it revealed that it has numerous capillary channels which form an intricate network that extends far over the ventral portion of the pleural investment of the lung and even penetrates the respiratory parenchyma and the central tendon of the diaphragm.

Under normal conditions, some of the filtrate from the lung probably passes into these capillaries, from them into the duct and from the duct into the aorta after the filtrate has had ample opportunity to give up much of its carbon dioxid and to take on some oxygen. The partial closing of the duct at or near the pulmonary artery should not interfere greatly with the elimination of the filtrate.

The right ductus arteriosus (ligamentum arteriosum in fig. 1) closes much earlier in life than does the left one. The difference in the time of closing of the two ducts is probably due to the difference in the angles which they form with the advancing stream of blood in the aorta. A fact not shown in figure 1, but in figure 3, is that the right duct, unlike the left one, lies snugly against the aorta for a considerable distance. Since the right duct turns the more sharply forward, deflection due to the stream of blood in the aorta should be the stronger in this duct, and the collapsing of its walls and the complete obliteration of it should occur in relatively early life. The right duct is in many cases closed entirely from the pulmonary artery to its point of union with the aorta while the left duct is still open. The system of capillaries associated with the right duct is seemingly as elaborate as that associated with the left one. The right duct, like the left one, is probably a part of the drainage system as long as it is completely or only partially open.

The ventral surface of each lung is also drained by numerous minute channels which communicate directly with the aorta or some of its ascending branches. The mouths of these branches are ordinarily hidden quite well by the extremely permeable endothelial lining of the aorta. Milk moves rapidly to the aorta when injected into the potential cavity between the central tendon of the diaphragm and the pleura of the ventral surface of a lung, and the rate of movement of the milk can be greatly increased by sending water through the aorta at various rates ranging between about two and nine meters per second. The cara cara eagle is especially suitable for this experiment because a natural cavity or pocket exists near the caudal end of the lung between the pleura and the tendon of the diaphragm (fig. 4). Milk injected into this pocket flows to the aorta principally in the membrane that separates the posterior thoracic and the anterior abdominal air sacs. In order to photograph the pocket filled with milk in a dead bird, it was necessary to prevent the rapid escape of the milk by coagulating it immediately with acetic acid. The capillary channels can be injected in some very fresh specimens by placing a liquid under great pressure in the aorta, and, if the connections (corresponding to the pulmonary ligaments in the mammal) to the aorta are cut, a very fine spray sometimes emanates from the aortic stumps of the capillaries. (This aortic spray was also observed under similar conditions in some mammals.)

The lungs of a dead bird may be rapidly dehydrated by sending water through the aorta at the rate of about seven meters per second, as was

done with a Victoria crown pigeon, a peacock, a crow, a duck, a crane and several other birds. The left lung only of a golden eagle was dehydrated by sending water through the aorta after the aortic arteries and capillaries to the right lung were ligated and clamped. The aorta and the ventral surface of each lung were moistened from time to time during the dehydration experiments so that the capillaries would remain open. Incidentally, all of the blood was deflected out of the ascending aortic, intercostal, inferior vertebral, musculo-phrenic and internal thoracic arteries of some of the fresh specimens.

## SUMMARY AND CONCLUSIONS

1. The greater number of the ascending branches of the descending thoracic aorta of the bird turn much more sharply forward than do the corresponding aortic branches in the mammal, and these branches in the bird accordingly constitute a much more efficient drainage system for the thoracic region than they do in the mammal.

2. It is essential that this system should be especially efficient in the bird because liquid constantly filters out of the pulmonary capillaries (as it does at expiration only in the mammal), the lymphatic system is either not very important as a drainage system for the thoracic region of the bird or it often fails to function when it is seemingly most needed and the filtrate does not have an opportunity to return to the circulation in the pulmonary capillaries (as it does in the mammal at inspiration). The bird lung does not inflate at inspiration.

3. So much of the filtrate from the bird lung is deflected into the aorta that the exhaled air of the bird contains considerably less moisture than does that of the mammal. Associated with this conservation of water is a conservation of sufficient heat to give the bird a normal body temperature a few degrees higher than that of the mammal.

4. The pulmonary filtrate is for a time in direct contact with the air that circulates in and immediately outside the respiratory tissue of the lungs, and it accordingly has ample opportunity to give up much carbon dioxid and to take on some oxygen before it is deflected into the aorta. This is probably the chief reason for the rather astounding efficiency of the small bird lung.

5. If the drainage system for the thoracic region of the bird is partially or totally blocked, the filtrate from the pulmonary capillaries collects in the lungs and air sacs, the normal conservation of water and heat no longer exists, the body temperature approaches or even falls below the normal temperature of the mammal and the bird may drown in its own plasma. Death is sometimes hastened due to a pulmonary hemorrhage.

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FORTY-SECOND ANNUAL MEETING

*Chicago, Illinois, March 27, 28, 29, 1930*

*The diffusion of water and of chloride through frog tissues.* EDWARD F. ADOLPH.

The rates of penetration of water into and out of tissues were studied by weighing the tissues at frequent intervals after transfer to a series of concentrations of NaCl. The tissues used were isolated entire skin, isolated gastrocnemius muscle, frog without skin, and normal intact frog. In addition the rates of diffusion of water *through* pieces of isolated skin and of lateral abdominal wall muscle were measured.

Within certain limits the changes of initial rates of diffusion with changes of concentration of NaCl in the medium were approximately linear. The slopes of these changes were employed to calculate diffusion coefficients. For the exchange into and out of tissues the formula of Stefan and Hill was applied; for the exchange through the membranous tissues the formula of Fick and Krogh. In the case of the living frogs the coefficients were calculated merely as a method of evaluating the rôle of the circulation in exchange of substances.

A gradient of one atmosphere of osmotic pressure was taken to be equivalent to a difference of 18 cc. of water in 22,400 cc. For skin and muscle the water coefficients, 0.4 to 1.5 sq. cm. per minute, were much greater than those known for fertilized sea-urchin eggs and human erythrocytes, and much below that for frog capillaries. For skin the chloride coefficient, $9 \times 10^{-5}$ sq. cm. per minute, was of the same order as the known lactate coefficient for muscle.

*The evaporation of frogs in relation to vapor tension.* EDWARD F. ADOLPH.

The object of the experiments was to find whether frogs lost water to the atmosphere in proportion to the vapor tension of the latter. Frogs were studied in still air and in a high air velocity, at constant temperature, over solutions of diverse vapor tensions. Evaporation was measured by weighing the frogs at intervals. In all atmospheres, even saturated ones, water was lost by evaporation; in saturated ones most of the loss was probably due to the slight gradient of temperature between the body and its surroundings, but part of it may have been due to factors such as the pH of the body surface which are known to influence the evaporation of gelatin blocks.

With a certain velocity of air motion the evaporation in low relative humidities was about six times as fast as in still air, due to the greater interchange of air near the skin surface.

Evaporation was rapid at first, partly because some water was already on the outside of the skin. In about two hours the rate became almost constant; it decreased very slightly and slowly during the next 24 hours.

Both the slope of the curve of water loss and the total loss in 24 hours per unit of body surface increased as the relative humidity decreased, but not exactly in linear proportion. It was, of course, impossible to measure the humidity at the very surface of the body. Particular study was given to the high humidities in the range in equilibrium with 0 to 0.4 M NaCl; but the differences of evaporation rates in this range were too small to evaluate accurately. It was therefore impossible to correlate vapor losses with the losses of water by diffusion when the frogs were immersed in salt solutions of the corresponding vapor tensions.

After desiccation frogs did not take up water from a saturated atmosphere but continued to evaporate in it. In water they swelled rapidly; the initial rate of this recovery was not related to the amount or speed of desiccation. Usually a slight excess of water was taken up and lost again before the original body weight was restored.

*Visceral reflexes from surface stimulation.* RAYBURN AUSTIN and W. J. MEEK.

The common belief that applications of various kinds must be placed on a specific skin area in order to influence a given internal organ has never been confirmed by actual experiment, so far as we know. The recently described kidney plethysmograph of Hargis has given an opportunity to demonstrate such a relation if it really exists. We have now prepared several animals with the kidney plethysmograph and have found that all surface areas are not equally important in inducing changes in kidney volume.

*The straight and diffused penetration of ultra-violet light into the human skin.* A. BACHEM and C. I. REED.

By use of various methods, the penetration of visible and ultraviolet rays from 576 to 236$\mu\mu$ through various specimens of human skin was determined. The various sources of error were studied or analysed carefully and special care was used to imitate conditions of treatment. It was shown that penetration is governed more by scattering than by true absorption. The distribution of the light through the various layers of skin was determined. The distribution of the light through the various layers of skin was determined and the practical limits established, to which the various parts of the spectrum reach. (Slides to illustrate.)

*Regeneration of fibres of the sympathico-adrenal system.* Z. M. BACQ[1] and S. DWORKIN.[2]

The occurrence of regrowth of pre-ganglionic fibres in the sympathetic nervous system has been studied by Langley (1897, 1900), by Kilvington and Osborne (1906), and by Lawrentjew (1925). In the fibres of the lumbar sympathetic chains Langley found a return of function as early as 35 days after sectioning. We have had occasion to study this question in cats in which both sympathetic chains had been removed in entirety from the stellate ganglia to the pelvis, about 1 year previously. In two animals out of three examined there was found physiological as well as anatomical evidence of regrowth of pre-ganglionic fibres. In both instances these

[1] Belgian Fellow of the C. R. B. Educational Foundation.
[2] Medical Fellow of the National Research Council.

fibres were traced from the adrenal glands, or the celiac ganglia, to spinal nerve roots. Electrical stimulation of these nerve fibres in one animal gave a marked acceleration in heart rate. In another cat, physiological evidence of medulli-adrenal innervation was furnished by greatly increased resistance to doses of insulin that induced convulsions in the same animal some months previously.

*The physiological action of an acid extract of normal thyroid glands with low iodine content.* HILLIER L. BAKER, CHARLES M. BACON, CLAYTON J. LUNDY and DAVID KLEIN.

This report covers over 100 observations made on dogs under local (novocain) and light ether anesthesia. Blood pressure records were made from the carotid artery; the electrocardiograph was used for rate estimations, and the pneumograph was used for observing respiratory rate. The tissues used were thyroid, kidney, suprarenal, liver, ovary, pancreas, duodenum, thymus, prostate, and muscle. Acid extracts of these substances were used and these extracts precipitated by varying strengths of alcohol. The iodine content of the thyroid extract was never more than 0.000016 gram per 1 cc. The extract represented 1 gram of tissue per cubic centimeter. Injections were administered intravenously.

The fraction not precipitated by our method showed a depressor action in the case of all the tissues examined accompanied by an increased heart and respiratory rate. The thyroid extract differed from all of the other tissues in that the precipitated fraction invariably produced a rise in blood pressure, a slowing of the heart rate, and an increase in the amplitude of respiration. It also produced a hyperglycemia, lowered the $CO_2$ capacity of the blood, and had no effect on blood calcium. These observations were made under local and light ether anesthesia.

The pressor effect persisted after section of both vagi, after atropine, and after section of the spinal cord just below the medulla. The slowing of the heart rate was not secured after vagus section or atropine.

The pressor effect of the thyroid fraction is not due to di-iodo-tyrosin or thyroxin, since neither of these in amounts several times as great as corresponded to the iodine in the fraction studied, caused a change in the blood pressure.

*Conclusions:* 1. An extract has been prepared from normal thyroid glands, with a low iodine content having the following properties: *a*, increases the blood pressure; *b*, slows the heart rate; *c*, increases blood sugar; *d*, lowers the $CO_2$ capacity of the blood; *e* has no effect on the blood calcium.

2. The rise in blood pressure was secured after vagus section, after atropine, and after the spinal cord had been transected just below the medulla.

3. The slowing of the heart rate was abolished by sectioning the vagi and the use of atropine.

4. Kidney, suprarenal, liver, ovary, pancreas, duodenum, thymus, prostate, spleen and muscle subjected to the same method of preparation did not yield extracts having the properties of the thyroid extract.

5. A depressor substance was found in the same fraction of every tissue studied including the thyroid and excepting the suprarenal gland.

6. The pressor and other properties of the thyroid fraction herein described are not due to thyroxin or di-iodo-tyrosin.

*Studies in chronaxie. I. Apparatus and theoretical considerations.* E. J. BALDES and J. R. LEARMONTH.

Measurements of chronaxie have been made with a portable chronaximeter designed for clinical use as well as for experimental work with nerve muscle preparations. Wide ranges of voltages are made available by using dry batteries. A voltage doubling switch facilitates the checking of the rheobase during the determination of the chronaxie.

Using a nerve muscle preparation measurements of chronaxie were made in which *a*, the voltage was equal to twice the rheobasic value, and *b*, the voltage was greater or less than the doubled rheobasic value. In the latter case the chronaxie was calculated from the equation

$$T = \frac{RC}{8}\left[\left(\frac{v}{v_0}\right)^2 - 1\right]$$

which reduces to the familiar value

$$T = \tfrac{3}{8}\, RC$$

when $v = 2\, v_0$.

*Factors producing high heart rates during decorticate sham rage in the cat.* PHILIP BARD.

Ablation of the cerebral cortex in the cat is followed by a behavior which is best described as sham rage. This activity occurs in fits during which there are signs of vigorous, widespread sympathetic discharge. Cannon and Britton[1] have shown that this form of emotional activity is accompanied by an increased secretion of adrenin. In fifteen experiments they found the average of the maximal rates of the denervated heart during activity to be 252 beats per minute, the average of the minimal rates during quiet 222 per minute; after double adrenalectomy the rate averaged 156 and was increased only four to eight beats during activity. It has been found that with cardiac nerves and adrenal glands intact the maximal rates attained during this activity are considerably higher than those reported by Cannon and Britton. This raised the question whether medulliadrenal secretion plays any rôle in the production of these more rapid rates. In an effort to answer this question the results given in the following table were obtained.

| CONDITIONS | HEART RATE PER MINUTE (AVERAGE FROM 15 EXPERIMENTS) | |
|---|---|---|
| | Minimal during quiet | Maximal during activity |
| Vagi, accelerators, and adrenals intact | 208 | 284 |
| Accelerators intact, vagi cut, adrenals out | 259 | 289 |
| Accelerators and adrenals intact, vagi cut | 264 | 305 |

In each of the forty-five experiments the cervical sympathetics were cut and the animals were fasting. When the adrenals remained intact one kidney was removed as a control procedure.

[1] Cannon and Britton. This Journal, 1925, lxxii, 283.

Taken together with the findings of Cannon and Britton these results indicate *a*, that accelerator nerve impulses are more potent than medulliadrenal secretion in producing the high heart rates; *b*, that even during the activity the vagi exert some inhibitory influence on the heart rate; *c*, that inhibition of vagal tone may allow, but cannot itself produce the high heart rates of the sham rage; *d*, that medulliadrenal secretion reinforces and supplements the action of accelerator impulses on the heart.

*Experiments on the mechanism of stimulation of end-organs for cold.* H. C. Bazett and B. McGlone.

A fold of skin (1.0 to 1.5 mm. thick) is obtained by suspension of the prepuce. Application of a cooled temporator (5 mm. diam.) to one surface, free of cold spots, elicits sensations of cold if cold spots are present on the opposite surface.

Comparison of the reaction times obtained from both surfaces with the temperature changes recorded by thermocouples on each surface indicates an approximate depth of 0.1 mm. for the cold end organ. This depth and the distribution agree with those, histologically determined, for "Krause," and confirm this end-organ as the cold receptor.[1]

The time, $x$, required to reach the threshold stimulus may be estimated by deducting from the reaction time the time necessary for the temperature changes to penetrate the 0.1 mm. depth, and 0.14 second for the nervous and motor processes. An estimate can then be made of the temperature changes involved in stimulation. With the stimulation from the nearer surface $x$ is a function of the rate of change, and sensation is induced at all rates of change above 0.2 to 0.3° per second and when the temperature at 0.1 mm. has fallen about 0.3° (min. 0.15° max. 0.5°). Stimulation of the opposite surface gives similar results but a change of 0.9° (min. 0.4° max. 1.25°) is usually required. In some experiments the necessary change is much greater (1.35° to 3.8°), and by no means constant. Greater values are particularly frequent, both in direct and indirect stimulation, in the presence of hyperemia. Stimulation, therefore, does not depend simply on the amount of change (Hering) since this value is constant only under certain conditions, nor on the rate of change (Weber), since $x$ may vary according to the surface stimulated, even when the rates of change are identical. The setting up of new spatial gradients might provide the most plausible explanation, but Ebbecke's hypothesis of differentiation between gradients at two levels would need modification to explain the results.

*The effect of pericardiostomy upon the mechanics of the circulation.* Claude S. Beck and William V. Cox.

Experiments were carried out on dogs to determine what effect, if any, exposure of the heart to atmospheric pressure had upon the mechanics of the circulation. The determinations that we considered important were: arterial pressure, venous pressure, and cardiac output. The method of determining the cardiac output was by means of the Fick principle.

Blood pressure determinations: Pericardium sutured to thoracic wall as a preliminary step. Femoral artery and jugular vein cannulated at the second stage of the experiment for pressure determinations. Immediately

[1] Williams, McGlone and Bazett. Amer. Journ. Med. Sci., 1929, clxxviii, 887.

upon incising the scar and opening the pericardial cavity to atmospheric pressure there occurred a fall in arterial pressure varying from 15 to 30 mm. of mercury, and a definite rise in the venous pressure. The fall in the arterial pressure was sometimes transient; the rise in the venous pressure was always sustained.

Cardiac output determinations: In one experiment the following results were obtained.

| DATE | OXYGEN CONSUMPTION | OXYGEN CONTENT, 1 CC. OF BLOOD | | ARTERIAL-VENOUS DIFFERENCE | CARDIAC OUTPUT | PULSE RATE |
|---|---|---|---|---|---|---|
| | | Arterial | Venous | | | |
| *1929* | *cc. per minute* | *volume per cent* | *volume per cent* | | *cc. per minute* | |
| February 25 | 105.81 | 15.98 | 10.87 | 5.11 | 2079 | 68 |
| March 1 | 116.72 | 18.93 | 12.45 | 6.48 | 1801 | 68 |
| March 6 | 107.84 | 15.40 | 10.43 | 4.97 | 2171 | 80 |
| March 18 | The pericardium was sutured to the thoracic wall | | | | | |
| April 1 | 103.14 | 16.43 | 11.06 | 5.37 | 1918 | 50 |
| April 9 | 89.07 | 17.09 | 12.44 | 4.65 | 1914 | 60 |
| June 4 | The pericardial cavity was opened to atmospheric pressure | | | | | |
| June 5 | 107.79 | 18.18 | 11.40 | 6.78 | 1579 | 72 |
| June 6 | 79.14 | 16.10 | 11.08 | 5.02 | 1577 | 92 |
| June 7 a.m. | 85.23 | 16.98 | 10.96 | 6.02 | 1414 | 96 |
| June 7 p.m. | 93.49 | 16.50 | 10.35 | 6.15 | 1518 | 96 |
| June 12 | 96.19 | 14.68 | 8.54 | 6.14 | 1567 | 92 |
| | The wound healed and the opening into the pericardial cavity had become closed | | | | | |
| June 19 | 95.14 | 14.99 | 9.96 | 5.03 | 1898 | 72 |

When the pericardial cavity was opened to atmospheric pressure the fall in cardiac output in different experiments varied from 15 to 30 per cent of the normal.

These changes in arterial pressure, venous pressure and minute volume output of the heart are produced by the pressure of the atmosphere exerted upon the intrapericardial structures. Positive atmospheric pressure acts much as does the collection of fluid in the pericardial cavity; it acts as an air tamponade. This mechanism merits consideration in the performance of operations upon the anterior thoracic wall, the pericardium, the heart or the mediastinum. In patients that are seriously decompensated it may constitute a vital factor.

*The inactivation of histamine.* C. H. Best and E. W. McHenry.

Histamine is rendered physiologically inactive by incubation under toluene with certain macerated tissues. The action is enzymatic in nature and the name histaminase is proposed for the inactivating substance. The optimum range of hydrogen-ion concentration is from pH 6.8 to 8.0. The optimum temperature is 38°C. Histaminase is heat-labile. Its action is not decreased by sodium, potassium, and phosphate ions, but it is inhibited by calcium ions and rendered completely inactive by 0.05 or 0.005 M potassium cyanide. The enzyme loses potency quickly in aqueous solutions but this loss can be prevented by addition of glycerol. Histaminase is present in relatively high concentration, in beef kidney, and in dog's kidney and intestinal mucosa. Moderate amounts are found in beef lung and in dog's lung, pancreas, spleen and skeletal muscle. Dog's

liver, heart muscle and blood contain relatively small amounts of the enzyme. An active stable preparation of the enzyme can be secured by defatting and drying minced beef kidney. Histamine added to blood perfused through dog's kidneys, is rapidly destroyed. Two hundred milligrams of histamine may be inactivated in four hours' perfusion. Perfusion of blood containing histamine through dog's liver under similar conditions, results in only a small loss. These perfusion experiments suggest that inactivation of histamine by the histaminase of the intestinal mucosa or of the kidneys, may be a normal physiological process.

*The average valence of the gelatin ion determined by means of a modified theory of membrane equilibrium.* R. BEUTNER.

From Donnan's theory, we can calculate the depression of the osmotic pressure of a gelatin solution, by the addition of a salt like NaCl. According to this theory this depression should not exceed a certain lowest limit; by the addition of ever so large an excess of NaCl the osmotic pressure should never become lower than one-half of the original magnitude in the absence of NaCl. Experimental observation shows that this is far from being true. As shown by the measurements of Loeb and others, salt addition depresses the osmotic pressure of gelatin frequently as low as $\frac{1}{10}$, sometimes to nearly $\frac{1}{50}$ of its original size. Manifestly such a deviation must be due to an insufficiency of the premises of the theory, probably to the too simple assumption of Donnan that the colloidal micellae carry single electrical charges. Theoretical considerations—to be published in extenso later—show that the *fraction of osmotic pressure which remains after the maximal lowering of the osmotic pressure by a salt excess amounts to* $\frac{1}{n+1}$ *where n signifies the valence of the colloidal ion;* hence the pressure is depressed to one-half for monovalent, $\frac{1}{3}$ for bivalent, $\frac{1}{4}$ for trivalent, $\frac{1}{11}$ for ten valent ions, etc.

From Loeb's measurements we can figure the osmotic pressure of gelatin chloride by adding to the observed values of osmotic pressure the counter-pressure which is due to unequal distribution of the $H^+$ = ions. This latter magnitude can also be figured from Loeb's data. By the addition of an excess of HCl practically the entire gelatin is transformed into gelatin chloride. For a 1 per cent solution of gelatin chloride the osmotic pressure is thus figured as 1500 to 1600 mm. water column. By the addition of excess of NaCl this is depressed to as low as nearly *23* mm.—all these data being taken from Loeb. The pressure is depressed, therefore, to about $\frac{1}{65}$ to $\frac{1}{70}$ hence the gelatin ion must carry a charge 66 to 71 times larger than a $H^+$ = or $Cl^-$ = ion. This can only be meant as the average charge since colloidal micellae are never of uniform size; gelatin moreover, is chemically inhomogeneous as proven by Northrop. Moreover, this result applies only to that sample of gelatin used by Loeb in his experiment and treated exactly the way he treated it. Nevertheless an approximate average value of this kind is of interest as it gives us some conception about the extraordinary size of colloidal ions.

*Basophilic or acidophilic staining of a dye as determined by an in vitro test.* R. BEUTNER and B. E. CAYWOOD.

Forty-three different dyes were used for testing the differential stain-

ability of artificial mixtures which contained either oleic acid or a fat soluble amine, and this was compared with the stainability of white blood cells. It was found in each case that a dye which was taken up, exclusively or preferably by a non-aqueous solution of a higher fatty acid, produced a differential stain on the nucleus of white blood cells. On the other hand, any dye with a preference for a mixture containing a fat soluble base preferably stains the cytoplasm and leaves the nucleus unstained, or but slightly stained.

Many nuclear stains like methylene blue are water insoluble bases. Evidently, they are taken up by a solution of oleic acid in a suitable solvent, forming, e.g., methylene blue oleate, which is oil-soluble and hence stains. However, there are also nuclear stains which are not bases, e.g., hematoxylin, a widely used nuclear stain which has all the characteristics of an acid. *This was also found to be taken up by the oleic acid oil mixture and not by the amine mixture.* Hence, in this case too, the test tube experiment with oil mixtures furnishes decisive evidence as to the biological staining power; one could foretell that hematoxylin must be a nuclear dye in spite of acidic properties in aqueous solution. Possibly hematoxylin has basic properties in oil solution.

*Correlation between time values and tension in skeletal muscle.* GEORGE H. BISHOP.

A green frog sartorius muscle clamped by the bone attachment at the pelvic end, fastened by a stout thread to a small windlass at the tendon end, and floated in a pool of Ringer's solution on a glass plate, may be freely stretched to different lengths. Stimuli are applied near the pelvic end and leads are taken, one from the middle and one from the distal end, to the cathode ray oscillograph, operated at a sensitivity of 50 meters per volt. All electrodes are fine steel wires, coiled to form light springs, and moving with the muscle. All are arranged longitudinally above the same fibers. A few fibers only need be stimulated, to obtain a record.

The muscle is first contracted to the shortest possible length by a strong stimulus, and then extended by steps, a record of threshold response being taken at each length, with rest periods between. The conduction time between given leads is found to be approximately constant as the length is increased, the *rate* of conduction thus *increasing* with tension, until slightly more than the normal length in the body is reached, after which the conduction *time* increases; the conduction *rate* stops increasing, and finally slows. The late slowing begins at approximately the tension which causes permanent distortion, that is, at the tension after which the tension-length curve fails to be reproducible.

The muscle conduction rate therefore shows a maximum at an optimum initial length of the muscle, quite as the tension developed in response to stimulation does, and within physiological limits of extension, the time of conduction tends to be constant with change of length. This contrasts with the condition in nerve, where the *rate* tends to be constant, the time varying with stretch.[1]

Other functions of muscle are being studied similarly.

[1] Carlson, A. J. 1911. This Journal, xxvii, 323.

*Some new data in the physiology of blood. II. Physiological etiology of anemia produced by pancreatic juice.* W. N. BOLDYREFF.

I made a report last year at the Congress of American Physiological Society presenting our data in experimental anemia, produced by introduction of excessive acid into the digestive tract. The present report deals with data of analogous experiments with the use of alkaline pancreatic juice.

Works of Delezenne have established the fact that dog's pancreatic ferments possess a highly developed hemolytic property. Wohlgemut and Karatygine have confirmed the same for human beings. All these authors have discovered that hemolysis is due to the effect of the proteolytic and lypolytic ferments and is increased in the presence of lecithin (Friedmann).

This property of the pancreatic juice may help to explain the cases of anemia when it is developed in patients with chronic achylia gastrica.

But experiments of the above mentioned authors were staged *in vitro*, the pancreatic juice getting mixed with blood in the test tubes. We have succeeded in making such experiments *in vivo* on fistular animals.

We can say that our data obtained on dogs are in complete agreement with those of the above mentioned authors. Here are two of many such experiments.

*Anemia produced by alkaline pancreatic juice absorbed from the small intestine (in fasting state)*

| | BEFORE THE INTRODUCTION (CONTROL) | AFTER THE INTRODUCTION OF PANCREATIC JUICE | | | | | |
|---|---|---|---|---|---|---|---|
| | | 1 hour | 2 hours | 3 hours | 48 hours | 96 hours | 168 hours |
| Dog "White." Weight 20,000. Isolated loop of small intestine (duodenum). January 10, 1930 | | | | | | | |
| Red cells | 7,100,000 | 6,950,000 | 6,100,000 | 6,030,000 | 5,620,000 | 6,330,000 | 6,930,000 |
| Leucocytes | 10,800 | 10,800 | 12,300 | 15,000 | 13,200 | 10,800 | 8,400 |
| Dog "Black." Weight 17,000. Ordinary fistula in the middle part of ileum. January 10, 1930 | | | | | | | |
| Red cells | 6,730,000 | 5,750,000 | 5,690,000 | 5,560,000 | 5,300,000 | 6,350,000 | 6,410,000 |
| Leucocytes | 12,600 | 14,700 | 15,600 | 16,200 | | 16,800 | 10,200 |

Both dogs had introduced, into the intestine, 100.00 cc. of the natural periodical mixture of pancreatic juice, bile and intestinal juice, obtained from another dog. This fluid has a little alkalinity (about 0.2 for $NaHCO_3$), and consists mostly of pancreatic juice (90 per cent).

Conclusion: The alkaline pancreatic juice possesses a hemolytic property and is capable of causing anemia, when absorbed into the blood from the intestine in abundant quantity.

*Experimental studies on factors altering intrapleural pressure.* SELLING BRILL and CHAUNCEY D. LEAKE.

By means of suitable trochars and rubber tubing, one may observe intrapleural pressures from both sides of the chest of normal unanesthetized dogs. The tubing may be branched so that both manometric and kymographic records may be obtained for intrapleural pressure changes from each side of the chest. With such an arrangement we have noted the effects on intrapleural pressure of 1, the injection of broncho-dilating and broncho-

constricting drugs in the unanesthetized animal; 2, anesthesia, and 3, manipulation of internal organs in the anesthetized dog. Normally, intrapleural pressure is subatmospheric. The administration of epinephrin or atropine is followed by an increase in intrapleural pressure so that it eventually may rise well above atmospheric pressure during expiration. After pilocarpine or eserine there is a decrease in intrapleural pressure below that noted normally. These facts are in accordance with the idea that intrapleural pressure varies inversely with the extent of obstruction offered to the passage of air through the bronchi. The lowering of intrapleural pressure after broncho-constriction is interesting in connection with the expiratory dyspnea and development of emphyse main asthma; inspiration is facilitated by the abnormally strong suction of the more negative intrapleural pressure, but expiration is impeded. During the induction of ether anesthesia the intrapleural pressure is greatly increased. During surgical anesthesia with ether, intrapleural pressure is subatmospheric but usually at a slightly higher level than normally. Manipulation of internal organs increases intrapleural pressure especially during expiration.

*Seasonal variations in survival of adrenalectomized animals.* S. W. BRITTON.

The effects of adrenalectomy were studied in the marmot (Arctomys monax). In the summer these animals rapidly succumb—usually within ten days—following removal of both adrenal glands. Indefinitely long periods of survival are however observed—extending at least over a few months—in animals operated on during the winter. The marmot is very active in summer, and its blood sugar at this time is high and normally between 120–190 mgm. per 100 cc. During the colder weather, although hibernation does not usually occur in captivity, these animals show much less activity, and their blood sugar is much reduced—particularly so in the case of adrenalectomized animals. The body temperature also reaches lower levels. The greatly protracted survival periods observed in winter in marmots deprived of adrenal tissues are possibly referable to reduced metabolic activities.

*Differential factors controlling the heart rate during emotional excitement.* S. W. BRITTON, A. HINSON and W. H. HALL.

An experimental analysis has been made of the various factors involved in regulation of the heart rate in the cat during intense emotional reactions attended by moderate muscular activity. Quick reflex changes in rate, i.e., those occurring within a minute or so after excitation, are mediated only through vagal, thoracic sympathetic, and adrenomedullary influences. Any one of these three influences may be eliminated without significant effect on the normal cardiac increments occurring during excitement.

The removal of *any two* factors normally concerned in regulating the activity of the heart is shown markedly to reduce its acceleratory ability—usually by about 50 per cent—following vigorous excitation of the unanesthetized animal. This incapacity persists throughout repeated stimulations extending over many weeks. If all three cardioregulatory factors are eliminated, negligible variations only in rate occur during excitement.

In over 100 cases in which normal animals were excited for one-and-a-half minute periods the average increase in heart rate was approximately

50 per cent over the basal level. Animals which were subjected to emotional stimulation every second day throughout an eight-weeks' period showed no diminution in cardiac response, and thus exhibited no tolerance towards the excitation.

*The energy expenditure in muscular contractions of varying strengths.* D. W. BRONK.

Recent work of Adrian and Bronk has shown that the variation in strength of a reflex or voluntary muscular contraction is in part due to a change in frequency of the motor nerve impulses. Weak contractions are maintained by asynchronous series of twitches in the several muscle fibre groups while strong responses are produced by a fused tetanus of the active fibres. In order to determine the economy of energy expenditure in contractions graded in this manner, the heat production and tension-time have been measured at various frequencies of stimulation. As the rate is increased, the economy or amount of tension-time maintained per unit of energy expended increases. This is largely due to a more complete fusion of separate muscle twitches, there being no increase in economy with frequencies of a value higher than that needed to produce complete tetanus. It may therefore be concluded that tension is maintained more economically in a strong contraction than in a weak one.

It is well known that a muscle stimulated at a frequency not quite sufficient to give partial summation will, as the result of continued stimulation, develop a fused tetanus at the same frequency. This altered character of the response is due to progressive fatigue. In order to determine the economy of maintaining tension throughout the development of such fatigue a muscle has been stimulated in nitrogen at a frequency of 4 or 5 a second for a period of some minutes and the rate of heat production and the tension-time developed have been measured. The results of these experiments show that a unit of tension is maintained with a lower expenditure of energy as the muscle fatigues. This fact is of probable importance in connection with long sustained contractions.

*The rôle of high temperature and panting in parathyroid tetany of dogs.* W. R. BRYAN and WALTER E. GARREY.

Extremely high temperatures may follow tetany in parathyroidectomized dogs and the resultant panting plays an important rôle in the symptom complex. In the early stages there may be marked fibrillary twitching of muscles without change in the normal blood picture, without elevation of temperature and without panting. On the other hand, there may be a slight decrease in blood pH, or a moderate elevation of temperature and definite panting without alteration of the blood picture. The onset of severe tetany however is accompanied by a sharp rise in temperature, violent panting ensues with overventilation, consequent increase in pH of blood and decrease in $CO_2$ tension. Due to these changes convulsions become more severe, the violent muscular activity now induces a blood pH well to the acid side of normal in spite of the evident overventilation with its tendency to produce alkalosis. Where the tetany is violent from the start the pH may also fall although there is an elevated temperature and severe panting.

If calcium lactate is injected at the height of tetany, convulsions cease in from two to twenty minutes, the temperature falls promptly but less

rapidly and panting persists from twenty to fifty minutes until the temperature is back to the prepanting level. These conditions cause an increase in blood pH which is due solely to overventilation, but it is never of a degree sufficient to reëstablish tetany in these animals or in normal animals, but under the conditions of hyperexcitability due to the low calcium level following parathyroidectomy the high temperature and increased pH resulting from panting are important factors in the severity and suddenness of onset of convulsions in dogs.

*The effect of irradiation by the mercury vapor arc on the activity of pepsin and trypsin.* DEA B. CALVIN.

Preliminary experimental results seem to indicate that ultraviolet irradiation causes a diminution in the enzymatic activity of pepsin and trypsin on gelatin and casein solutions. The course of digestion has been followed by Northrop's modification of Sorensen's formol titration method for free amino groups. Temperature and other factors have been controlled. The enzyme solutions to be irradiated have been placed in quartz cells. Enough solution was used to fill the cell completely and the cell was covered with a quartz plate to exclude the air. A constant quantity was used each time giving a constant surface area and depth of solution. The distance of the cell from the mercury arc was the same for each period of irradiation. Data in hand indicate that the decrease in activity is most pronounced during the early stages of digestion while the final point of equilibrium is practically the same for the control and irradiated samples of enzyme. Pepsin appears to be more stable to this treatment than trypsin. Further work of a quantitative nature is in progress.

*On the mechanism of the action of hydrostatic pressure on striated muscle.* MCKEEN CATTELL and DAYTON J. EDWARDS.

In a previous report we showed that the augmenting action of hydrostatic pressure on the tension developed by striated muscle in response to a single stimulus is proportional to the magnitude of the pressure employed up to 1000 lbs. per square inch. Using a similar technic, we have now investigated the effects of greater pressures up to 6000 lbs. per square inch. In one series of ten experiments, in which the tension was recorded at 1000, 1500, and 2000 lbs., it was found that the points fell in an approximately straight line, with an average increase of 28.6 per cent at 2000 lbs. In another series of seven experiments, in which the pressure was increased in steps of 1000 lbs., the maximum stimulation, averaging 35.3 per cent, was reached at 3000 or 4000 lbs. At the higher pressures the amount of stimulation produced by equal increments of pressure became less and less. At 5000 lbs. the stimulation was much less or entirely absent, the average for the group being 7.7 per cent. A pressure of 6000 lbs. without exception resulted in a decrease in the power of developing tension, the seven experiments averaging 66.2 per cent of the control value obtained with the muscle at atmospheric pressure. This effect is a reversible one and closely resembles the depression caused by lower pressures in the fatigued or chilled muscle.[1] For the lower pressures the duration of the twitch (contraction plus relaxation) is not markedly augmented, averaging about 12 per cent for each increment of 1000 lbs. pressure up to 4000 lbs., and this is roughly

[1] Cattell and Edwards. Science, 1930, lxxi, 17.

the range of pressure which gives an augmentation in contraction. In the higher pressures which cause depression there is a striking prolongation of the duration of the twitch, the change amounting in these experiments to 60 per cent between 4000 and 5000 lbs. and 170 per cent between 5000 and 6000 lbs. As soon as the muscle is returned to atmospheric pressure the duration of the twitch returns to the control value. In other conditions, in which relatively low pressures cause depression there is also a prolongation of the contraction-relaxation period. A pressure of 1500 lbs. applied to a fatigued or cold muscle, causing a depression around 50 per cent in the tension developed, commonly prolongs the response over 100 per cent. These facts suggest that the reversal of the augmenting action occurring at the higher pressures may be due to an influence on the viscosity of the muscle. At a low temperature or in the fatigued muscle (high pH) this effect is reached at lower pressures. This hypothesis is further supported by the observation that a muscle under the combined influence of low temperature and high pressure, in which a single stimulus results in but a barely preceptible response, will respond to a tetanic stimulus with a tension which is slowly built up but which is of the same order as that obtained at atmospheric pressure. In cardiac muscle a reversal of the augmenting action of pressure at high degrees of compression, low temperatures or fatigue has not been observed, and this is instructive in the light of the very much slower processes of contraction and relaxation obtaining in this tissue and consequently where an increase in viscosity would play a less important rôle.

*The effect of partial vs. complete interruption of the proprioceptive pathway upon the form of respiratory contractions.*[1] HELEN C. COOMBS.

Recent work upon the neuro-muscular respiratory mechanism of adult and young animals has led to some new observations.

1. I have recently shown[2] by hemisection of the posterior corpora quadrigemina and unilateral division of the dorsal roots of the spinal nerves that the mechanism—nuclei and paths—in the corpora quadrigemina concerned in respiration, is ipsilateral. I have now been able to check this observation in another way. If, after decerebration, the mid-brain is split longitudinally[3] in the mid-line, the respiratory contractions continue without any alteration, although such a section generally abolishes the decerebrate rigidity which appears after removal of the cortex.

2. After division of the dorsal spinal nerve roots of a limb, there is a certain inertia fling, or lack of adequately controlled movement, while after a partial lesion of the proprioceptive path, e.g., the cerebellum, there is hypermetry with forced uncontrollable movements; in the one case, there has been a complete loss of all afferent impulses, proprioceptive and exteroceptive, from the limb; in the other case, there is a break in only one portion of the proprioceptive pathway. In the neuro-muscular respiratory mechanism in adult animals, while division of the dorsal spinal nerve roots of the intercostals, or of the vagi, or both, causes a slowing of respira-

[1] The cost of animals for this work was met by a grant from the American Association for the Advancement of Science.

[2] Coombs, H. C. Science, lxxi, 1930, 136.

[3] Pike, F. H., C. A. Elsberg, W. S. McCulloch and M. N. Chappell. Arch. Neurol. and Psychiat., 1930 (in press).

tion with a decrease in magnitude of the costal or diaphragmatic contractions respectively, the respiratory rhythm remains regular although the rate and depth may be altered; whereas, if section is made behind the posterior corpora quadrigemina, the respiratory contractions, particularly of the diaphragm, may become erratic and lose their precision and regularity of form. It would appear from the somewhat uncontrolled nature of these contractions as if such a section had interrupted only a portion of the proprioceptive pathway concerned in the maintenance of respiratory movements.

3. In kittens and puppies 3 to 5 days old, section of both vagi is followed by a slowing in rate and irregular contractions of the diaphragm. Costal contractions remain relatively unaffected. The vagus control over the diaphragmatic movements appears to be acquired earlier ontogenetically than the control exercised by the afferent fibers of the intercostals.

*The anorexia characteristic of so-called vitamin B deficiency: is it due to a lack of the antineuritic factor, the antipellagra substance, or both?* GEORGE R. COWGILL, H. S. ROSENBERG and J. ROGOFF.

Symptoms of a deficiency of what has hitherto been designated vitamin B include among other things an anorexia of very interesting and subtle nature. Recently it has become clear that what has been called vitamin B really consists of at least two physiologically active substances: the antineuritic principle ($B_1$ or vitamin F), and the antipellagra factor ($B_2$ or vitamin G). It is of interest to determine whether the anorexia just referred to is due to a shortage of the antineuritic component alone, the pellagra-preventive substance only, or both.

Using the technique developed in this laboratory for the study of vitamin B physiology in the dog, we have made tests 1, with a fuller's earth adsorbate of the antineuritic vitamin F;[1] 2, autoclaved yeast as a source of the antipellagra vitamin G, and 3, fuller's earth alone for control experiments.

*Every trial with the adsorbate* (nine in all) resulted in a prompt restoration of the desire to eat. In the first two tests with autoclaved yeast there was a suggestion of a positive result. The yeast was therefore autoclaved again. In four tests with this twice-autoclaved material there was *no effect* on the anorexia. Administration of fuller's earth alone was *likewise without effect* on this characteristic loss of appetite. When the adsorbate was given following negative tests with both the yeast and fuller's earth, however, there was a prompt restoration of the desire to eat. We feel justified in concluding that this anorexia characteristic of so-called vitamin B deficiency is indicative of a lack primarily of the antineuritic principle ($B_1$, vitamin F). Inasmuch as the basal diet contained some of the antipellagra factor as an impurity chiefly of the casein employed, it is possible that this component of the vitamin B complex plays some rôle; if so, then the amount necessary as compared with that of the antineuritic substance is relatively small. Further experiments, using more highly purified diets, and designed to elucidate this point, are in progress.

[1] Furnished by Dr. C. N. Frey of the Fleischmann Company Laboratories, New York.

*A study of possible mechanisms for gall-bladder contraction and evacuation.* LATHAN A. CRANDALL.

Ivy and Oldberg[1] have shown that the presence in the duodenum of acid or fat liberates a hormone, cholecystokinin, which causes contraction and partial evacuation of the gall bladder. In the present study an attempt has been made to evaluate other possible mechanisms concerned.

Dogs with permanent esophageal and gastric fistulae were prepared. After visualization of the gall bladders of these animals by tetraiodophenolphthalein they were subjected to sham feeding with egg-yolk, meat, and water; in no case did such sham feeding result in any change in the size or shape of the gall-bladder shadow. In other animals shielded electrodes were placed in the vagi just above the diaphragm and the gall bladders visualized by tetraiodophenolphthalein or by filling them with iodized oil; stimulation of the right or left vagus in such animals produced no change in gall-bladder shadow.

In two dogs with pouches of the entire stomach the gall bladders were visualized by tetraiodophenolphthalein, and the gastric pouches were lavaged with egg-yolk; one of these dogs showed no change in gall bladder shadow on two occasions. In the other animal the gall bladder emptied $\frac{1}{2}$ to $\frac{2}{3}$ of its contents after $2\frac{1}{2}$ hours of lavage.

In five dogs the gall bladders were filled with brominized ethyl ester of olive oil (Brominol light) which has a viscosity of 5 compared with water. The viscosity of gall bladder bile has been found to average about 2. The viscosity of lipiodol is 90. Brominol light has been found to be no more irritating to serous membranes than is lipiodol.[2] Gall bladders filled with this oil all showed a partial spontaneous emptying within 5 hours even though the dogs were given nothing by mouth.

It is concluded that there is no evidence that either psychic nervous influences or the vagi are concerned in gall bladder emptying in the dog. Ivy and his collaborators[3] have shown that cholecystokinin is present in the pyloric mucosa. The fact that in one dog egg-yolk in the isolated stomach caused evacuation of the gall bladder indicates either that this may be a normal mechanism for liberation of the gall bladder hormone, or that a local sympathetic nervous reflex may be implicated. The spontaneous emptying of radio-opaque oil of low viscosity may conceivably be due to irritation from the oil, although no evidences of irritation were found in the gall-bladders. Another possible explanation is that a washing out of the contents of the gall bladder and its replacement by liver bile takes place.

*Lactation as influenced by different sources of protein in the diet.* R. G. DAGGS.

A study was made of the effects of different sources of protein in the diet upon the production of milk in dogs and rats in an attempt to gain light upon the much debated question, "Can lactation be permanently affected by dietary means?"

Liver is much better than either egg or round steak as the chief source

[1] Ivy, A. C. and E. Oldberg. This Journal, 1928, lxxxvi, 599.
[2] Crandall, L. A., P. Holinger and E. L. Walsh. To be published.
[3] Ivy, A. C., G. Kolster, H. L. Lueth and G. E. Drewyer. This Journal, 1929, xci, 336.

of protein in the diet of lactating dogs. The pups showed the greatest percentage weight increase when the mother was fed liver from the time of conception through the fifth week of lactation and the mother secreted the most milk as well as the best milk, as shown by quantitative collection and analysis of the milk during the fifth week of lactation.

Egg and kidney appeared to be the best sources of protein for lactating rats as judged by the growth rate of the young. A study was made of the lactation-promoting factor present in these substances. Extraction of the dried meat or egg with cold 70 per cent alcohol or ether does not remove the vital factor. Precipitating the meat protein from a 0.05N sodium hydroxide solution seems to render it much less satisfactory for lactation. An attempt is being made to isolate the vital factor.

*Changes in our conceptions of localization of certain functions in the brain.* WALTER E. DANDY.

From a series of cerebral operations of varying character several conclusions have been reached:

1. It is possible to remove all of the right cerebral hemisphere above the basal ganglia with no appreciable disturbance of mentality; this procedure has been carried out on several occasions for the removal of extensive tumors in the right hemisphere.

2. On one occasion both frontal lobes have been completely extirpated for the treatment of a bilateral frontal brain tumor, and following this there has been no appreciable disturbance of mentality. The patient is perfectly oriented as to time, place and person; the memory is unimpaired; he reads, writes and conducts mathematical tests accurately; his conversation is seemingly perfectly normal.

Furthermore, by the excision in other cases of the left occipital lobe and of the lower third of the left temporal lobe, we can be sure that none of these regions are responsible for intelligence. The intellect, therefore, is concerned with the remaining portion of the left cerebral hemisphere and is doubtless closely related to the speech mechanism.

3. It has been found after ligation of the anterior cerebral artery on the left side that consciousness is completely and forever lost. This does not result when the same vessel is ligated on the right side. There is, therefore, within the limits of the distribution of this vessel an area specifically concerned with consciousness. The same result follows when this vessel is occluded at the middle of the corpus callosum, indicating that the seat of consciousness is posterior to this point in the vessel.

4. The entire body of the corpus callosum may be split in the midline without any appreciable disturbance of function. This structure is, therefore, eliminated from participation in the important functions which hitherto have been ascribed to it.

*The metabolism of glucose administered to the fasting dog.* MARGARET DANN and WILLIAM H. CHAMBERS.

The effect of fasting on the rate of combustion of carbohydrate has been studied in the following observations on two emaciated dogs. The glycogen stores of one animal were depleted by exercise on a treadmill during a fasting period of 22 days. On 4 successive days the basal metabolism was determined in the respiration calorimeter, then 50 grams of glucose were ingested, and again the respiratory metabolism was determined for the 2nd, 3rd, and 4th hourly periods.

Of the first 50 grams of glucose ingested 6.7 grams were excreted in the urine, the R.Q. rose from 0.72 to 0.74, and only 0.22 gram per hour was oxidized during the 3 hours of observation. The blood sugar concentrations were 50 mgm. per cent before the glucose ingestion and 350 mgm. per cent 4 hours afterward. The following day less than 2 grams of sugar were excreted, there was less hyperglycemia, the R.Q. increased from 0.72 to 0.86, and 2.73 grams per hour of glucose were oxidized. On the 2 succeeding days there was progressively a greater increase in the R.Q. and more glucose oxidized.

After the animal had received for 10 days a mixed diet which maintained its body weight but did not change its basal metabolism, the same amount of glucose resulted in a rise in R.Q. from 0.92 to 0.98 and the oxidation of about 5.2 grams per hour. Similar data have been obtained from another dog which had fasted for one month and was about 50 per cent under weight.

These results show that the marked hyperglycemia, which a number of investigators have reported when glucose ingestion takes place after prolonged fasting, is also associated with a definite impairment in the ability to oxidize glucose as well as to retain glycogen in the liver, both of which latter conditions are associated with lack of insulin. It likewise appears that the relation between the height of the specific dynamic action of ingested glucose and its concentration in the blood may be modified by the intensity of the insulin producing function of the pancreas.

*Fatigue in skeletal muscle in relation to the frequency of stimulation.* HALLOWELL DAVIS and PAULINE A. DAVIS.

The soleus muscle of the cat was stimulated indirectly, and action currents and tension of the isometric contraction were recorded. The nerve was cut central to the point of stimulation to avoid reflex activity. The animal was decerebrate or under light ether anesthesia and circulation through the muscle was active.

The fresh soleus muscle gives a (nearly) fused tetanus in response to stimuli at a frequency of 20 per second. Increases in the frequency of stimulation above this cause small increases of tension. In a fatigued muscle fusion may occur at rates of less than 10 per second.

Continued stimulation at a given frequency causes a fall in tension slowly approaching a definite "fatigue level." Action currents diminish in like manner. The more rapid the stimulation the lower is the level attained. When a low level has been reached with rapid stimulation an abrupt diminution of frequency will cause an immediate increase in tension, accompanied by increased action currents. The tension level and the size of action current characteristic of the slower rate are asymptotically approached from below. The lower level of activity associated with the more rapid stimulation is not due to a "Wedensky" inhibition of some fibers.

The behavior of the action currents closely resembles the "equilibration" phenomenon in nerve. Increase or decrease in the action currents is accompanied by a parallel change in mechanical tension, and a causal relationship is suggested. The fall in tension under rapid stimulation seems to be due to a prolongation of the relative refractory period and the associated period of subnormal electrical response.

Tonic contraction of skeletal muscle need not involve rotation of activity of fiber groups, but may be accounted for in terms of the steady state of fibers contracting in response to a slow frequency of nerve impulses.

*Embolic decerebration with lycopodium and lampblack mixture.* W. T. DAWSON.

Lycopodium spores are particularly suitable for producing experimental cerebral embolism because of their uniform diameter of about 32 mu. The incorporation of lampblack enables one to see what regions have been embolized, the vessels blocked appearing as black threads or cords or spots, according to the section. The components of the mixture are: acacia powder, 0.7 gram, intimately mixed dry in a mortar with 0.5 gram lycopodium and then with 1 gram commercial lampblack; to this is added 10 cc. water, saline or Ringer's solution, at first gradually, and the whole well mixed. The mixture is very thick but the spores settle rather rapidly. The mixture is given a final stir and the calculated amount drawn up into a hypodermic syringe, a large gauge (e.g., 20) needle being put on last. The animal having been anesthetized, and one carotid tied, and the other provided with two loose ligatures, the injection is made into the second carotid between the loose ligatures and toward the brain. After a few seconds the ligatures are tied, and the needle then withdrawn. The mixture will jam in the syringe or needle if the injection is not made quickly after filling the syringe, and it is well to have the piston coated with petrolatum. The suitable dose for the dog has been found to be about 0.1 to 0.125 cc. per kilo. Usually the material does not go into the basilar artery sufficiently to stop the respiration. Ordinarily extensor rigidity occurs, but it is often partial, i.e., affecting only two or three limbs. Both elevation and fall of temperature have been observed, in different cases. Death ordinarily occurs in a few hours. The respiration may be slackened or made more rapid. The embolism is often uneven as can be seen grossly in some of the brains. The tongue and other parts in the head are usually also plainly embolized, sometimes on one side only. Gross inspection of sections of brains hardened in formalin shows that the observed effects of the injection are in general what would be expected in the light of current teaching as to the functions of various parts of the brain, e.g., red nucleus. The method seems to be not readily applicable in the cat and rabbit, the arteries of the circle of Willis probably not being sufficiently large in diameter for the injection mass to flow freely always into the cerebral hemispheres, especially the contralateral one.

*Effect of ovarian extract on motility of rhythmically contracting uterus.* E. P. DURRANT.

The rhythmic contractions of excised rat uterus in oxygenated Tyrode's solution were recorded from the entire horn and from ovarian and cervical ends separately. While contracting normally the segments of uterus were subjected to the action of 0.1 per cent, 0.2 per cent and 0.3 per cent of different commercial ovarian extracts. Of thirteen animals thus studied, all but one responded with diminished activity up to complete inhibition with the largest dosage. Similar dosage had no effect on segments of intestine from the same animal.

*Effects of ovarian extracts on voluntary activity of white rat.* E. P. DURRANT.

Eighteen mature female white rats were kept in self-registering revolving cages until the cyclic variations in activity associated with oestrus were clearly shown on graphs prepared from the daily runs. One half were then injected subcutaneously with 0.2 cc. of a pharmaceutical ovarian extract each day. The other half were similarly treated with distilled water. The treatment, continued for twenty-four days, was without effect on the rhythm of activity but produced a marked disturbance in the vaginal mucosa.

*The mechanism of adrenalin glycemia.* SIMON DWORKIN[1]

Recent work (Cori and Cori)[2] on the function of adrenalin in carbohydrate metabolism indicates that the adrenalin hyperglycemia is due to some inhibitory action on peripheral sugar utilization, rather than to conversion and release of stored glycogen. These conclusions have been adduced from experiments with rather large amounts of adrenalin, administered subcutaneously and assumed to be absorbed at a slow rate.

We have studied the effects of much smaller quantities of adrenalin—0.001 to 0.004 mgm. per kgm.—injected intravenously, in single doses. Most of the experiments were carried out in cats under amytal anesthesia; a few in normal cats, and in dogs. Provided that the blood sugar level to start with was below 130 mgm. per cent, it is rare to find, with the amounts of adrenalin used, an increase in blood sugar beyond the renal threshold, or above 70 mgm. per 100 cc.

In many experiments we have used the denervated heart as an indication of the presence of adrenalin in the blood stream. When, under these conditions, small amounts of adrenalin are injected intravenously (or liberated by splanchnic stimulation) the duration of the increase in the heart rate never exceeds 10 minutes; often the peak is reached 1 minute after the injection. The maximal blood sugar level is reached usually in less than 3 minutes; and the glycemia may outlast the heart rate increase by 40 minutes to 3 hours. If adrenalin, as such, has produced the glycemia, it must have exerted its action in a few minutes—a view hardly compatible with the idea that adrenalin raises blood sugar by decreasing sugar utilization. There is, of course, the possibility that the adrenalin, quickly removed from the circulation, remains for a long time in the peripheral tissues; proof of this has not been furnished. In any case, it is obvious that the effects from small, intravenous doses of adrenalin are different from those obtained with the amounts referred to above. The fact that splanchnic stimulation gives results similar to those obtained with the small amounts indicates that such quantities more nearly correspond to the physiological activity of the adrenal glands.

*The effect of cooling upon chronaxie and refractory period of nerve.* S. DWORKIN[3] and M. FLORKIN.[4]

The effect of temperature upon the chronaxie and refractory period of nerve was studied in the sciatic-gastrocnemius preparation of Rana pipiens.

[1] Medical Fellow of the National Research Council.
[2] Cori and Cori. Journ. Biol. Chem., 1928, lxxix, 343.
[3] Medical Fellow of the National Research Council.
[4] Fellow of the C. R. B. Educational Foundation.

A stretch of nerve about 1 cm. in length was placed within a cooling chamber; stimulating electrodes were applied in the centre of the cooled region, while the muscle contraction was used as an index of nerve response. Chronaxie was measured by means of the Lapique chronaxiemeter and by condenser discharges. Refractory period was determined with a Lucas type pendulum, using two successive induction shocks of equal strength, just above the threshold value.

At room temperature, our figures for chronaxie of nerve are in the neighborhood of $0.3\sigma$, while those for refractory period vary from 1.5 to $2\sigma$; these values correspond to those found in the literature. During cooling both processes are affected by temperature in accordance with the Arrhenius equation. Between 25° and 15°C. we obtained a temperature coefficient of 1.6; between 20° and 10°C., it was about 2; below 15°C. the coefficient was 3. Under the conditions of our experiments, it would appear that between chronaxie and refractory period of nerve there exists a definite ratio of 1:6. This relationship holds good for all temperatures from 25° to 2°C.

*The in-vivo autolysis of pancreas.* JAMES C. ELLIS and LESTER R. DRAGSTEDT.

The effect of in-vivo autolysis of the pancreas was determined by occluding the blood supply of varying amounts of the organ in five normal dogs. Two died in depression in 36 hours. Three survived the initial toxemia and eventually recovered. In each case there occurred a fall in the concentration of blood chloride and a rise in the non-protein and urea nitrogen.

A bacteriologic examination was made of the pancreas of apparently normal healthy dogs and in seven out of ten experiments an anaerobic, non-spore bearing, Gram positive bacillus was found. In each case the pancreas was removed under ether anesthesia and with strictest aseptic precautions. After cultures were made, the pancreas was placed in the general abdominal cavity of a second animal and the effect of its autolysis on the blood chemistry and survival noted. The relation of the presence of this organism in the apparently normal pancreas to the toxemia of pancreas autolysis is discussed.

*Further studies in cardiac hypertrophy.* J. A. E. EYSTER.

One hundred and ninety subjects, with prolonged athletic history were submitted to physical examination with particular reference to heart size. The methods used were 1, orthodiagraphy in the frontal and lateral planes; 2, orthodiagraphic location of the anatomical apex; 3, presence or absence of a visual apex, and if present its location; 4, the presence or absence of apical or precordial "heaving;" 5, murmurs or other signs of cardiac abnormalities. These subjects were divided into three groups, 83 male university students of an average age of 22.8 years, 83 women university students of an average age of 21.8 years, and 24 professional athletic coaches of an average age of 32.4 years. All subjects were selected on the basis of duration and continuity of athletic history extended back through the primary and secondary schools. The results in these subjects were compared with 100 cases of normal subjects selected without reference to athletic history and with 100 cases of compensated organic heart disease. The conclusion is that a prolonged athletic history is associated with no evidence, by the methods employed, of cardiac dilatation and hypertrophy.

If athletic activity results in cardiac dilatation and hypertrophy, the change is small, beyond our present methods of determination, and not comparable with that associated with organic heart disease.

*The oxygen consumption of muscle under the influence of sugar and other contracture producing substances.* W. O. FENN.

Measurements have been made of the excess oxygen consumption of frog sartorius muscles in chemical contracture. The tension-time developed in match muscles or similar muscles was also determined. On the whole the ratio of these two quantities, i.e., the excess oxygen per unit length of muscle per unit of tension-time was found to be of the same order of magnitude as for electrical stimulation. This applied to the contraction by acetyl choline as well as the contraction of HCl. All these substances therefore act in part at least upon the same mechanism. High concentrations of caffeine 1 per cent, quinine 1 per cent, HCl 0.01M, NaOH 0.01M and NaCl 6 per cent, cause cessation of the oxygen consumption after the initial increase. The interpretation is occasionally complicated also by a permanent increase in either oxygen consumption or tension or in both.

Isotonic sucrose causes an initial contraction accompanied by electronegativity of the muscle and a temporary rise in the oxygen consumption. This is inhibited by 0.113 per cent NaCl but not by 0.05 per cent $CaCl_2$ or 0.08 per cent K Cl. Superimposed upon this effect is a more or less prolonged increase in the oxygen consumption accompanied by electropositivity of the muscle which is inhibited by small amounts of K Cl, $CaCl_2$, lactic acid, potassium sulphate and phosphate and other substances without causing a return of excitability. Spontaneous non-irritability due to failure to soak the muscles in Ringer's solution resembles the non-irritability due to sucrose and other sugars in being accompanied by a high oxygen consumption and in that isotonic potassium salts cause no contraction, or only a small contraction and a diminution rather than the usual increase in oxygen consumption.

*The absence of "all-or-none" in the heat production of skeletal muscle after direct stimulation.* ERNST FISCHER.

The responses of a muscle to direct and to indirect maximal stimulation are not strictly identical. In a certain number of muscles one always finds a distinctly larger heat production after direct stimulation, while other muscles respond to both kinds of stimuli with approximately the same energy change. An exact comparison, however, is practically impossible, as the maximal tension developed is a well defined value only after indirect stimulation, while after stimulating the muscle directly the determination of the maximal stimulus is to a certain extent an arbitrary one. The curve for the tensions obtained after direct stimulation plotted against the strength of the stimuli does not show a sudden transition to a straight line parallel to the abcissae, but takes an asymptotic course. The curve for the heat productions plotted against the strengths of the stimuli corresponds to that for the tensions only up to a certain strength of stimulus (which may be called the "critical" stimulus); beyond this point the curve for the heat production rises sharply, i.e., there occurs an increased heat production without increased tension. The strength of the "critical" stimulus shows no constant relation to the strength of the "maximal" stimulus. Sometimes the critical stimulus is ten or more times as large

as the maximal, while in other muscles the critical stimulus can be even weaker than the approximate maximal stimulus. The latter muscles show markedly different heat productions for the same tensions developed in response to different types of direct electrical stimuli (induction shocks, condenser discharges). Since an excessively strong single shock produces an increased tension by setting up several impulses (a short tetanus), it is difficult to find by using single shocks a significant relation between the higher strengths of stimuli and the heat productions. Experiments with short tetani, however, gave heat productions proportional to the logarithms of the stimulating energies.

*The spleen and blood concentration.* B. A. FLESCHE, L. B. WINKELSTEIN, and F. H. SCOTT.

It is well known that great alterations in the number of corpuscles or hemoglobin content of the circulating blood can take place in a very short time. Since the work of Barcroft and his co-workers on the contractility of the spleen and the high hemoglobin content of its pulp, there has been a tendency to attribute to the spleen all of these effects on blood concentration. The effect of some procedures producing sudden changes in concentration of the blood have been investigated with and without the spleen. It is possible to get marked alterations in the concentration of the blood after the spleen has been removed. Barcroft and Florey have shown that the blood is concentrated in the spleen by the removal of the plasma. Other organs besides the spleen do the same. The spleen is only one factor in the alteration of the blood. The factor of movement of fluid in and out of the blood is, in most cases, far more important. One must not forget that it is just as easy to get a very great dilution of the blood in a few minutes, as it is a concentration.

*The balancing of antagonistic reflex effects in a spinal center.* A. FORBES, H. DAVIS and E. LAMBERT.

We have studied the isometric contractions of the vasto-crureus muscle (knee extensor) of decerebrate cats in response to simultaneous stimulation of the contralateral sciatic nerve, which reflexly excites this muscle, and the ipsilateral peroneal or popliteal nerve, which inhibits it. The results illustrate the dominance of the flexion reflex over the crossed extension reflex, as shown by complete inhibition of the latter by stimulation of either peroneal or popliteal nerve, shown by the action current to be barely maximal, or even submaximal. In the case of weaker submaximal stimuli, increasing the excitatory background against which the inhibitory stimulus operates not merely adds more motor neurones to the number previously involved, but robs the inhibitory stimulus of the power to inhibit as many motor neurones as it did when acting against a weaker excitatory background.

Post-inhibitory rebound is probably due to stimulation of excitatory fibers of relatively high threshold in the inhibitory nerve, contributing little to the action current, whose activity is masked by the inhibition. We likewise find evidence for inhibitory fibers in the contralateral sciatic nerve which is typically excitatory, these fibers also having a relatively high threshold.

These results, as well as all others with which we are acquainted, may be interpreted either according to Sherrington's hypothesis of central

excitatory and inhibitory states, or upon a theory based entirely upon the known properties of peripheral nerve. No final decision can yet be made between these two working hypotheses.

*Multiple ovaries and oestrus.* J. L. FRIEDMAN and L. B. NICE.

Twenty-one female white rats each received an ovary from two other white rats so that each animal had four ovaries—its own pair and one from each of two others. The oestrus of these rats was studied by the smear method before and after transplantation. After transplantation the cycles were found irregular and much more frequent than before. The activity of these rats does not correspond closely to the oestrus cycles.

*The ending of the axon action potential and its relation to the recovery process in nerve.* H. S. GASSER and J. ERLANGER.

(The paper will soon appear in full in this JOURNAL.)

*Further evidence of the invalidity of the all-or-none principle.* SAMUEL GELFAN.

That graded responses to graded stimuli can be elicited in the single fibres of the frog's retrolingual membrane, was shown by the writer in a previous paper. Further evidence is presented demonstrating the invalidity of the all-or-none principle in the sartorius and gastrocnemius of the frog as well. With circulation intact, the stimuli were localized to single fibres by means of very small non-polarizable micro-electrodes. Photographic records were made by the mercury droplet method of Pratt and Eisenberger.

*Further studies upon vagal inhibition of the turtle atrium.* ARTHUR S. GILSON, JR.

Work previously reported[1] has been continued and extended. Auricular electrograms and myograms have been obtained from hearts *in situ* and excised with one or both vagi intact. The vagus trunk was stimulated high in the neck region. The results reported are not affected by careful cutting of the sympathetic branches.

Electrographic and myographic records show a definite effect of vagus stimulation at a stimulation strength well below that required to produce slowing or interference with sino-auricular conduction. These effects are in accord with those reported by earlier workers who found inotropic effects of vagus stimulation not referable to chronotropic changes. The low threshold effect includes diminution of the height of auricular contraction, quickening of systolic time, and shortening of the duration of the electrical response but no significant slowing either of the frequency or of sino-auricular conduction. There may even be slight increases of conduction rate and frequency of beat. With increasing strengths of stimulation these first effects appear in increasing degree. At a certain sharply defined threshold there begin to appear slowing of the heart, interference with sino-auricular conduction or both. As previously reported by by us these effects at first do not stop the sinus or the atrium although with increasing strength or time of stimulation stoppage occurs. The

[1] Gilson and Irvine-Jones. 1929. This Journal, xc, 361 (Proc. xiii Int. Physiol. Cong.).

threshold values as recorded do not have absolute significances but an indication of the ranges involved may be found in citation from a typical experiment. A Harvard, cored induction coil with one dry cell was used. Stimulation was done faradically, platinum electrodes 2.5 mm. apart being used. With coil separation of 11 cm., threshold for stimulation of two nerves to skeletal muscles of a turtle used was with the coil at an angle of about 80°, the primary (inotropic) effects appeared with the coil at an angle of about 45°, chronotropic and dromotropic effects appeared with the coil practically horizontal.

It thus appears that there is not merely an incidental but a primary and definitely demonstrable separation between the negative inotropic effect of vagus stimulation and the later severely depressing effects of the vagus upon heart rate, conduction and irritability to external stimuli.

*Effects of summation in the peripheral and central retina of the human eye.* RAGNAR GRANIT.

Four circular test patches of 1° of diameter placed symmetrically on the circumference of an imaginary circle of 3° or 4° of diameter were shown to give a higher fusion frequency with an intermittent stimulus than each of the patches tested singly. This was evident within a wide range of intensities and the effect increased with the brightness of the test patches, provided the centre of the four stimuli fell on a spot situated 10° out toward the periphery. With central fixation and the patches falling on the edge of the fovea there was a very slight increase of the fusion frequency when the four were taken together over the value obtained with each of them singly.

There is no absolute difference between the fusion frequency for a peripheral and a central stimulus. With large areas stimulated the fusion frequency is generally higher in the periphery; with small areas, in the centre.

The limits of validity of the Ferry-Porter law, $n = k \log I + k_1$, where $n$ = fusion frequency, $I$ = intensity and $k$, $k_1$, are constants, are also shown to be dependent upon the size of the area stimulated.

The retina is not only a sense organ but also a "true nervous centre" (Cajal) and the degree of interaction, individualization or concentration of the impulses within its system of neurones will have to be considered in order to give an explanation of results such as the ones mentioned above.

*Reflex cardiac reactions through the phrenic nerves as afferent pathways.* CHARLES W. GREENE.

Physiologists have paid little attention to afferent constituents of the phrenic trunks although clinicians since Hippocrates have known that acute pain in diaphragmatic pleurisy was projected to the neck with contraction of neck muscles.

Far reaching clinical contributions were made by Capps who in recent years has explored both surfaces of the diaphragm and adjacent structures on conscious human patients by an ingenious method of mechanical stimulation. He circumscribed areas over the pleural surface of the diaphragm and the lower third of the pericardium in which stimulation produced pain referred to the right shoulder and neck, while regions of the parietal pleura and of the extreme outer margin of the diaphragm were interpreted as local pain. The middle portion of the peritoneal surface of the dia-

phragm produced referred shoulder pain while adjacent areas were interpreted as pain of nearby areas of the abdominal wall. He was able to exclude the inner wall of the pericardial sac, the cephalic portion of the pericardium and the pulmonary surfaces.

In 1922 Felix demonstrated that the phrenic distribution to the pleural surface of the diaphragm contained sensory fibers of somatic and also sympathetic origin. He also showed connecting trunks between the inferior cervical ganglia and the phrenic trunks in the neck. Several authors have given evidence that stimulation of the phrenic produced vasomotor changes in the lung itself, and reflexes affecting the amplitude and rate of respiration have been recorded. Apparently the phrenic nerves contain a generous supply of sensory elements.

In our experimental determinations of the distribution and character of nerves influencing the heart and the coronary arteries we have explored the phrenic nerves. The method of study of the coronary flow is based on measurements of the output from the coronary sinus through a Morawitz-Zahn cannula and recorded by a water manometer. The results have been controlled by simultaneous blood pressure determinations and by an arterial constant pressure head.

In the dog under ether we have determined the following types of phrenic reflex.

1. Stimulation of the afferent ends of either phrenic, both being sectioned and the vagi poisoned with atropine but all other nerves intact, we find distinct augmentation of respiratory volume with increase in respiratory rate as manifested by the movements of the chest wall.

2. If the blood pressure is not equalized then there is usually a reflex rise of blood pressure.

3. The heart rate is strongly augmented by afferent stimulation of the phrenics. This rate may increase any amount up to 30 per cent and more.

4. The coronary flow from the coronary sinus has varied under the reflexes from phrenic stimulation. The majority of our experiments showing decided dilatation of the coronary vessels with increase in volume flow per minute. In one or two animals the sensitivity of both the cardiac accelerator and the coronary dilator mechanism has been very great. In a certain proportion of animals the phrenic reflexes are slight or absent. In a few instances we have observed measurable decreases in coronary flow. These have occured when the vagi were sectioned, and when all other factors are taken into consideration have been interpreted as definite vasoconstrictions which cannot be ascribed to vagus pathways. It is quite possible that the thoracic dilator pathways sometimes contain coronary constrictors.

*Can the production of carbohydrate from fat be demonstrated in the perfused liver of the dog?* D. E. Gregg.

Dogs were fed on a normal diet or on a diet of thick cream from four to thirty days. In three dogs, four days previous to the perfusion, phlorhizin was given. In two cases adrenaline was added to the perfusing fluid. In all eighteen experiments were performed.

The livers were placed in an incubator kept at body temperature and were perfused from one to three hours with oxygenated, defibrinated or heparinized blood, either through the portal vein or through the portal vein and hepatic artery simultaneously.

The livers of the fat-fed dogs showed an appreciable glycogen content,

the average of fifteen livers being 0.716 per cent. In all preparations there is a marked variation in the fatty acid, glycogen and free sugar content of the various lobes of the liver.

Chemical analysis of such perfusions brings out an increase in true blood sugar, blood non-fermentable reducing substance, lactic acid and urea. The liver glycogen, free sugar and fatty acids decrease. The fatty acids of the blood are not altered.

An attempt to balance the total fatty acid and carbohydrate in the perfusion system shows a decrease in both constituents. Under the conditions of the experiments there is no demonstrable conversion of fatty acids to carbohydrate.

*Glycogen formation in rats.* ESTHER M. GREISHEIMER and OLGA H. JOHNSON.

Adult rats were kept on McCollum's stock diet I for a three weeks observation period, and then fed the test diets for three weeks. During both periods the food consumption and water intake were measured.

The control rats remained on the stock diet during the entire six weeks. This diet has a caloric value of about 3.88 cal. per gram. About 25.4 per cent of the caloric value is derived from protein, 20.3 from fat, and 54.3 from carbohydrate. The test diets used are shown in the following table, the figure in parenthesis indicating the per cent of the total caloric value derived from each constituent.

| DIET | CASEIN | SUCROSE | LARD | SALT 185 | CAL. PER GM. |
|---|---|---|---|---|---|
| A | 14.5 (13.1) | 81.6 (86.9) | 0 | 3.9 | 3 77 |
| B | 30.0 (12 4) | 0 | 80 (87.6) | 8 2 | 6 95 |
| C | 95.0 (87.8) | 0 | 5 (12.2) | 4 2 | 3 53 |

The rats on each diet increased in weight during the test period. The caloric intake increased slightly on the sucrose and lard diets, but decreased on the casein. The water intake increased on the casein, and decreased on the sucrose and lard diets.

The absolute liver weights were closely comparable on the test diets. However, if liver weights were considered in relation to body weight, the high lard diet produced a lighter, the casein a heavier liver, than the stock diet.

The glycogen contents were as follows: 4.49 $\pm$0.19 per cent on the stock diet; 6.03 $\pm$0.18 on sucrose; 1.79 $\pm$0.12 on lard; and 2.02 $\pm$0.15 on casein. The increase on sucrose is significant. The decrease on both lard and casein is significant.

By a study of the caloric intake during the last twelve hours of life, and the glycogen content of the liver, we found that rats form glycogen most easily from sucrose, and with difficulty from lard and casein. Expressing the ease of glycogen formation in terms of efficiency, with the stock diet as unity, the sucrose diet efficiency may be represented as 1.85, the lard as 0.44 and the casein as 0.61.

*Further studies on the cardiac output of normal man.* ARTHUR GROLLMAN.

Determinations of the cardiac output of normal individuals have been extended to a number of physiological conditions in an effort to further elucidate certain problems connected with the circulatory system.

The effects of anoxemia as produced by inspiring air of low oxygen content at normal barometric pressure, were studied in comparison with the results previously obtained by the author at high altitudes. Anoxic anoxemia was found to cause an immediate increase in the cardiac output when the oxygen content of the inspired air was reduced beyond a certain critical point which corresponded to an oxygen saturation of the arterial blood of about 83 per cent. Further reduction of the oxygen content of the inspired air below this point resulted in a marked increase in the cardiac output.

The effect of inspiring *carbon dioxide-air* mixtures, of voluntary forced breathing, with and without change in the carbon dioxide content of the alveolar air, were also investigated. The inspiration of air containing more than 6 per cent of carbon dioxide resulted in an increase in the cardiac output. Voluntary forced breathing for short periods of time resulted in similar increases.

Because of their wide use in everyday life, the effects of caffeine, tobacco and alcohol on the circulatory system, with particular reference to the cardiac output, was also studied.

Further substantiation of the accuracy of the Foreign Gas Principle, as applied in the method employed in the above mentioned investigations, was obtained by comparing the results of measurements by the use of *acetylene* with the *triple extrapolation method* based on the Fick principle. The results by the two methods agreed within the limits of the experimental errors. The previously established value of the normal basal cardiac output (2.2 liters per minute per square meter of body surface) and the previously demonstrated independence of the cardiac output to postural changes were substantiated by the results of both the acetylene and triple extrapolation methods.

*Blood flow and intrathoracic blood volume, as determined by the injection method and checked by direct measurement in perfusion experiments.* W. F. Hamilton, J. W. Moore, J. M. Kinsman and R. G. Spurling.

The assumptions upon which is based the injection method for determining the cardiac output and the width of the active intrathoracic vascular bed, are as follows:

1. That the mathematical relationships between time and concentration which holds during the early part of the first circulation, continues to hold during that part of the first circulation which occurs after the dye has begun to re-circulate.

2. That dye does not diffuse from the pulmonary vascular bed during its first passage.

3. That the time concentration relationships can be determined with a fair degree of accuracy.

By experiments in which the lungs and left heart were perfused, these assumptions were checked and found to hold.

1. The time concentration curve shows the postulated linear relationship between time and log. concentration which continues, in the absence of re-circulation, indefinitely.

2. The dye can all be recovered after perfusion, if vital red is used. Phenol-tetraiodo-phthalein diffuses in varying amounts, out of the lung capillaries and should not be used.

3. The flow, calculated from the results of the injection method, checks

within −1.2, −3.8, −5.4, −6.3, +12.2, +1.6 and +14.6 per cent in several experiments.

4. The width of the intrathoracic vascular bed, as represented by the product of the mean circulation time and the flow, falls within the rather variable figures for this quantity derived from the dilution of the vital red by an unknown amount of blood in the chest of the perfused dog plus the known amount of blood in the tubes and vessels of the perfusion apparatus.

*The energy metabolism of pregnant rabbits.* HANCE F. HANEY.

The work here reported deals first with a comparison of the total energy metabolism of the mother animal just before parturition with the total of mother and young early in the post partum period; and second with the determination of the metabolic rate of the pre-partum animal on a surface area basis. The following procedure was used to determine the surface area of the pre-partum mother. The weight of the new-born young was subtracted from that of the mother just before parturition, and the resulting figure used in determining the surface area of the mother. The surface area of the new-born young was then calculated and added to that of the pre-partum mother, giving a figure which represented the combined surface area of mother and fetuses. The total number of calories produced by the pregnant organism was divided by the figure representing combined surface area of mother and fetuses, and the result taken as calories per unit of active protoplasmic mass of the pregnant organism just before parturition.

The apparatus used was of the modified Haldane open circuit type. The general laboratory average metabolic rate for normal adult rabbits was 2.61 calories per kilo per hour, and 32.7 calories per square meter per hour, when the surface area formula, $10.8 \times (\text{W. in Kgm.})^{\frac{2}{3}}$ of Rubner for the rabbit without ears was used.

It was found that the total metabolism of rabbits is not markedly changed at parturition; that is, the total heat production of the mother just before parturition is about equal to that of the mother and young combined just after parturition.

If the fetus be considered as a separate organism and its surface area plus that of the mother be used in computing the metabolism, no increase in rate is evident during pregnancy.

During the early post partum period the metabolism of the rabbit, both per unit of weight and per unit of surface area, is considerably above normal.

*A further study of the hormone of the adrenal cortex.* F. A. HARTMAN and K. A. BROWNELL.

The potency of our extracts has been tested by their ability to prolong the lives of completely adrenalectomized cats. As controls we have injected eight cats every four hours with a small quantity of 0.9 per cent NaCl solution and seven cats twice daily with 6 cc. of the same solution. These cats lived an average of 6.2 days ranging from 1.5 to 10.3 days. In addition 20 animals were adrenalectomized but not injected. They lived an average of 11.5 days ranging from 2 to 35 days.

The average of thirty-five control cats was 9.2 days.

We have reached the following tentative conclusions in regard to the cortical hormone.

Keeping the whole adrenal on ice or at 35°C. for eighteen hours before extraction seems to destroy the hormone or produce a deleterious substance

which offsets its value. Preserving the gland on ice in the absence of oxygen is more satisfactory. Acetone, apparently, does not precipitate the hormone. The hormone seems to be destroyed by heating to 80°C. for 5 minutes and by dilute alkali. The activity is preserved better by separating the cortex from the medulla and by preservation at a low temperature, if the tissue is to be kept some time before extraction.

*The respiratory quotient on high fat diets.* ESTELLE E. HAWLEY, CARROLL W. JOHNSON and JOHN R. MURLIN.

A series of 14 experiments on two subjects, E. H. and C. J., was carried out on three ketogenic-ketolytic ratios 1.5:1, 3:1 and 4.1:1. Respiratory quotients were obtained every hour and a half after the noon meal, for a period of nine hours. The Benedict Universal respiration apparatus was used and the subject was connected to it through a face mask. The machine was checked with alcohol for each experiment.

Blood sugar, blood acetone and $\beta$-hydroxybutyric acid; urine N, $NH_3$, acetone and $\beta$-hydroxybutyric determinations were made.

The subjects ate a weighed and measured diet over the entire period. Determinations were carried out only on 4 and 5 days of diet.

The R.Q.s obtained were corrected for N, $NH_3$ and total acetone excretion. In spite of these corrections many of the quotients remain below 0.70 on the high fat levels.

*The effect of fibers of different types in the vagus and sympathetic nerves on the heart.* PETER HEINBECKER.

Investigations by the author have demonstrated three fiber types in the vagus and sympathetic nerve trunks yielding three separable components of potential. The first type (larger relatively thinly myelinated) has been shown to be a visceral afferent. Proof of this among other things rests upon investigations of the thoraco-abdominal ganglion of the turtle vagus. The potential arising from this fiber type conducts through the ganglion without delay in both directions, and produces alteration in the nuclei of the cells in this ganglion which are of a readily recognizable type. Such changes afford a basis for the establishment of their sensory character. The second fiber type (small thinly myelinated) is efferent in character, as is also the third or unmyelinated type. Proof for the efferent character of these two types has been obtained through a functional analysis of the superior cervical ganglion of the turtle by means of the cathode ray oscillograph.

Stimulation of the first fiber type, as would be anticipated, has no effect on the heart. Stimulation of the second fiber type in the vagus shortens the systolic time and lowers the amplitude of contraction of the turtle auricle without change in heart rate. Stimulation of the third type (unmyelinated fibers) slows or stops the heart. With this slowing there occurs further accentuation of the shortening of systolic time and diminution in amplitude of contraction. This is considered to be due to a greater effect from the second or thinly myelinated fiber type whose maximum stimulus lies within the stimulus range of the unmyelinated fibers. Stimulation of the unmyelinated fibers in the sympathetic produces an acceleration of heart rate and a relief of partial inter-regional block. There is here evidence of a definite antagonism of action by similar fibers (unmyelinated) of the parasympathetic and sympathetic nerves. In animals

other than the frog the inotropic effect resultant from stimulation of the sympathetic fibers to the heart has been slight. In keeping with this the small thinly myelinated fibers in these nerves in animals studied other than the frog are very few in number.

*Effect of the sympathetics on glycogen content of muscle.* RAYMOND C. HERRIN.

This is an inquiry into the possible relationship of the sympathetic nervous system to the glycogen content of skeletal muscle. Investigations were conducted along two lines of procedure. First, we studied the effect of unilateral stimulation of the lumbar chain with strong induction shocks, using urethane-ether as an anesthetic. Results obtained with six rabbits were quite variable. Stimulation in case of three animals resulted in a lesser glycogen content on that side, while in other cases the opposite difference or none was observed. Second, after unilateral sympathectomy of the lumbar chain and a month's recovery allowed, the thigh muscles were analyzed for glycogen by Pflüger's method, the reducing sugar being determined by the Folin and Wu colorimetric method. Of the results obtained with nine rabbits, eight showed significantly lowered glycogen content on the sympathectomized side. The hamstring group showed 28 to 50 per cent less glycogen, whereas the quadriceps showed practically no difference or differences only up to 6 per cent. Similar experiments were conducted with dogs. Of the muscles on the operated side the hamstrings showed a reduction of from 3 to 6 per cent whereas the quadriceps showed about 14 per cent. It is suggested that the sympathetic nervous system exerts a trophic influence to aid in maintaining a sufficient glycogen supply to the tonic muscles, the hamstrings in the rabbit and quadriceps in the dog.

*Substitution of lecithin for raw pancreas in the diet of the depancreatized dog.* J. M. HERSHEY.

The depancreatized dog receiving insulin fails to survive more than eleven months on a diet of lean meat and sugar. Symptoms observed shortly before death are decreased urinary volume and sugar excretion, the presence of bile pigments in the urine, jaundice, hypoglycemic reactions and general apathy. At autopsy there is found an extensive infiltration of fat into the liver, which is the only organ seriously affected. The liver may contain up to 35 per cent fat which has an iodine number of about 65.

The addition of raw pancreas to the diet prevents the occurrence of this condition. The present work was begun with the hypothesis that the condition was caused by a failure in fat metabolism, rather than a failure of digestion produced by the absence of the pancreatic enzymes. It was thought that the condition might be prevented or cured by adding lecithin to the meat-sugar diet.

In eight dogs in which the typical condition appeared, the addition of ten grams of lecithin daily to the diet restored the animals to an apparently normal condition. The urine became free of bile and increased in volume. The sugar excretion became greatly increased. In the one dog in which it was followed the urinary nitrogen did not change. Two dogs killed for examination after one year of lecithin feeding had normal liver glycogen and liver fat. After one year of lecithin feeding the lecithin in the diet of a dog was replaced by suet. The animal died in a short time in the con-

dition described above. Three dogs receiving lecithin died during the course of the experiments with symptoms very suggestive of vitamin deficiency.

It is concluded that the pancreatic enzymes are not essential to life in the dog and that lecithin can be successfully substituted for raw pancreas in the diet of depancreatized dogs.

*Effect of stimulation of hypothalamus on blood glucose.* H. E. HIMWICH and A. D. KELLER.

It has been established that puncture of the hypothalamic region of the brain may be followed by glycosuria. We have endeavored to study this phenomenon more closely by stimulation of various parts of the brain with induced currents. The glucose contents of the samples of blood drawn before, during, and after stimulation were determined by the method of Hagedorn-Jensen.

In all, 41 cats under amytal anesthesia were studied. The hypothalamus was stimulated 26 times, and the characteristic result was a rise in blood glucose. However, the more profound the anesthesia, the more intense the current necessary to produce this rise. When the cats were deeply under amytal in 4 observations no rise of glucose was obtained. With light anesthesia there is usually an increased concentration of the blood glucose following even a very weak stimulation. In contrast with these results are others in which stimulation of the various lobes of the cerebral hemispheres was followed by no change in blood glucose. Stimulation of the cerebellum and medulla also produced no rise in blood glucose, unless asphyxial changes supervened.

Section of the brain-stem through the hypothalamus raised blood glucose immediately when the anesthesia was not profound.

*The physiology of the ileo-cecal sphincter.* JOSEPHINE HINRICHSEN.

A study of the innervation and observation of the normal control of the "ileo-cecal sphincter" of the dog was made in a series of thirty-five experiments. A true anatomical and physiological sphincter was found to be present.

On the basis of results obtained from electrical stimulation of nerves, it can be said that in the dog the splanchnic nerves and the pelvic nerves are motor to the ileo-cecal sphincter. The vagus nerve is chiefly inhibitory. Somatic sensory nerves have no direct detectable effect on its activity. The reflex control was studied by distention of various portions of the digestive tract. Distention of the ileum and colon resulted in contraction of the sphincter. That the intrinsic innervation is important is brought by the fact that contraction of the sphincter results on distention of ileum or colon when the extrinsic nerves have been cut.

Injection of epinephrine, $\frac{1}{10}$ cc. 1:10,000 and of pilocarpine, 1 to 2 cc. 1:10,000 gave marked contraction.

Observation through a cecal fistula reveals an active sphincter which allows material to pass intermittently in 2 to 10 cc. amounts over extended periods of time.

Fluoroscopic study in normal unanesthetized dogs brings out the following points: A small quantity of barium milk placed in the distal ileum through a fistula is not immediately evacuated into the colon; but within fifteen minutes after ingestion of food, complete evacuation into the colon

occurs. This is not a psychic effect, but appears to be due primarily to a reflex elicited by distention of the stomach or duodenum.

*The contralateral flexion reflex and terminal rebound phenomena in spinal cats.* J. C. HINSEY and S. W. RANSON.

In cats which had recovered from the immediate effects of transection of the spinal cord at levels varying from L 3 to T 4 (1 to 23 days after the operation) the spinal reflexes were tested in two ways. While the limbs were still intact the animal was suspended on a sling with the legs hanging pendent and the reactions in both hind legs to pinching the toes of one hind foot were carefully observed. In many of the animals these tests were repeated on several different days. Finally the animals were decerebrated and prepared for recording the reflex responses of the tibialis anterior and gastrocnemius muscles to stimulation of various nerves. The ipsilateral and contralateral tibial nerves at the ankle were the ones chiefly employed. The stimuli were rapid make and break induction shocks of moderate intensity.

Both when the reflexes were studied in the intact limbs and when the movements of the tibialis anterior and gastrocnemius were recorded separately the contralateral flexion reflex was observed somewhat more frequently than the crossed extension reflex. It is usually followed by extensor rebound after the end of the stimulus. Weak stimuli seemed more effective than strong ones in eliciting the crossed extension reflex. When the limbs were intact lightly touching a foot would sometimes cause extension of the other leg when vigorously pinching the toes together would cause contralateral flexion.

We have some evidence that crossed flexion is more readily elicited from the tibial at the ankle than from the saphenous nerve, more readily with moderately strong than with weak stimuli, and more readily with rapid than with slow interruptions of the primary circuit (50 per second and 4 per second).

Many of the terminal rebound phenomena described by Graham Brown have been seen and studied.

*Influence of follicular and corpus luteum hormones on the uterine endometrium of rabbits and monkeys.* FREDERICK L. HISAW, R. K. MEYER, SAMUEL L. LEONARD and H. L. FEVOLD.

Extracts of sow corpora lutea yield a crystalline and a non-crystalline fraction which produce characteristic physiological reactions in experimental animals. The crystalline fraction causes relaxation of the pelvic ligaments of castrate female guinea pigs while the non-crystalline fraction promotes several reactions generally accredited to the corpus luteum, two of which are the developement of the progestational endometrium in the uterus of castrate rabbits and the premenstrual endometrium in the uterus of castrate monkeys. Relaxation as well as progestational and premenstrual changes in the uterine endometrium require the action of both the follicular and corpus luteum hormones, neither being able to elicit positive results in castrate animals when given alone. Neither can the corpus luteum hormones promote and preserve the relaxed, progestational, or premenstrual conditions over an extended period in the absence of the follicular hormone. The corpus luteum hormone alone will not prevent menstruation in a castrate monkey previously treated with follicular hor-

mone nor prevent the return of the progestational endometrium of a rabbit to a castrate condition. These reactions seem to be of a "one two" nature, i.e., the follicular hormone must act first, followed by the corpus luteum hormone and both must be present to prolong the effect. The crystalline fraction of the corpus luteum which produces the relaxation of the pelvic ligaments of the guinea pig, bears a qualitative relationship to the follicular hormone, while the non-crystalline fraction which produces the uterine changes, bears a quantitative relationship to the oestrus producing hormone. That is, large doses of follicular hormone do not inhibit the action of the relaxative hormone, but in the case of the non-crystalline fraction large doses of oestrin will prevent its action on the uterus.

*Respiratory exchanges during work and recovery.* F. A. HITCHCOCK and W. C. MCNELLY.

Experiments have been carried out on twelve men ranging in age from 17 to 42 years. In each case the respiratory exchanges were determined by the Tissot method, 1, while the subject sat still on the bicycle ergometer; 2, while he worked on the ergometer for a period of about five minutes, at the rate of 0.851 Calories per minute, and 3, for a recovery period (spent in sitting still on the ergometer) of about ten minutes, divided into two approximately equal parts. Six of the subjects were athletes who were in training while six were not of the athletic type and were not in training. During the work period the oxygen consumption of the athletic group increased 232 per cent while that of the non-athletes increased 244 per cent even though the work done by the athletes was slightly greater than that done by the non-athletes. The athletes accomplished this increase in oxygen consumption by increasing the ventilation rate 148 per cent and increasing the per centage of oxygen absorbed 33 per cent. With the non-athletes the ventilation rate increased 173 per cent and the per centage of oxygen absorbed 26 per cent. The average muscular efficiency of the athletic group was 20.82 per cent while that of the non-athletic group was 19.57 per cent. During the recovery period the oxygen consumption fell off more rapidly than the carbon dioxide production. The result was a sharp rise in the respiratory quotient. The average respiratory quotients for all twelve subjects were as follows: sitting 0.822, exercising 0.837, 1st recovery 0.930, 2nd recovery 0.855. The respiratory quotient of the total excess metabolism was 0.910. This apparently indicates a preference of the muscles for cabohydrates and seems to be in agreement with the theories advanced by Hill and Meyerhoff.

*The respiratory quotient of resting mammalian muscle as shown by the eviscerated spinal cat.* LAURENCE IRVING and H. C. FOSTER.

The R.Q. of the decapitated cat when maintained by artificial ventilation is about 0.73 with the stomach empty. After evisceration the R.Q. rises. This rise has been considered as an indication that muscles are restricted to the use of carbohydrate, but it has also been shown that the rise in R.Q. is accompanied by a loss in blood and muscle $CO_2$.

By carefully reducing the rate of artificial ventilation after evisceration, we have been able to maintain an R.Q. close to that before evisceration. When the R.Q. does not depart from that before evisceration, the steady blood $CO_2$ content shows that there is no overventilation. If the blood $CO_2$ falls or increases, the R.Q. is increased or diminished.

The eviscerated cat is essentially a surviving muscle preparation, so that its R.Q. represents that muscle is not restricted to the utilization of carbohydrate for resting metabolism, but makes use of the same material as the whole animal. Even when the blood sugar is maintained at a high level by continuous infusion of glucose, the R.Q. is not necessarily one; so that even with an abundant supply of sugar the muscles do not use sugar exclusively.

*The effect of cholecystokinin on the human gall bladder.* A. C. IVY, G. E. DREWYER[1] and B. H. ORNDOFF.

A "purified" solution of cholecystokinin (1860 preparation) was prepared and shown to be neither toxic in the dog nor antigenic in the guinea pig. It was free of vaso-dilatin and active in the dog in 3 mgm. doses. From 25 to 30 mgm. of this material has been injected intravenously at ten minute intervals into five normal subjects and three dispensary patients in which gall-bladder disease had been suspected. The gall bladder was visualized with phenoltetraiodophthalein; of the five normal subjects, all but one manifested some degree of gall-bladder evacuation when injected every ten minutes for one hour. In one the evacuation was complete, in the other three it was partial. Two of the five felt "light-headed" about one hour after completing the injections, which sensation disappeared within the course of 30 minutes, no other symptoms being complained of. Only three doses were given at ten minute intervals to the three dispensary patients. Two of the three showed a definite decrease in the size of the gall-bladder shadow, one of them having a pericholecystitis. Further work on the human will not be undertaken until the product is further purified.

*The antagonism between the carotid and vertebral circulations with respect to the control of the heat regulating centers.* F. JELSMA.

When the vertebral arteries of the dog are heated as they pass from the brachial arteries to the spine, 1, the peripheral vessels constrict; 2, restlessness occurs and sometimes *shivering*; 3, if sufficiently heated, hyper-ventilation and dyspnea occur (followed sometimes by apnea and periodic respiration). A similar picture is produced by *cooling the carotids.* (Cf. cooling the base of the brain.)

When the vertebral arteries are chilled 1, peripheral vasodilatation occurs; 2, the dog exhibits marked repose; 3, respiration becomes shallow; 4, vomiting may occur. A striking duplication of these effects may be obtained by *heating the carotids.*

Now the two arterial regions may be treated simultaneously with opposing temperature influences, without *directly* changing the caloric balance of the body. In this case the rectal temperature tends to swing in the same direction as the medulla, and the opposite way from the basal ganglia. The vasomotor and muscle effects are all in accord with this result. In other words, cooling the carotids, like cooling the basal ganglia, can raise the body temperature; heat applied to the brain acts as an antipyretic.

The most consistent results are obtained when the basilar artery has been ligated or cut, some days in advance. This procedure separates in a

[1] Eli Lilly Fellow.

general way, two functionally opposed nervous fields, and checks the temperature spread from one to the other.

The above various central effects of temperature seem best explained by recognizing heat always as a stimulant and cold as a depressant. Hans Meyer's para-sympathetic "cooling" center would be located in the basal ganglia, inhibiting his sympathetic "heating" center, the medullo-pontine region. The latter region would include Sherrington's shivering center. Shifting the stimulus to the inhibitory centers above, produces the same effect as depression of the medullary region.

*The rôle of the ventricle in the filling of the heart.* LOUIS N. KATZ.

Some observations made on the isolated, perfused turtle ventricle led to the conclusion that the heart under these conditions develops a pressure less than that in the reservoir early during its relaxation, especially when the heart is vigorous and the diastolic size large. This became evident when the ventricle was allowed to fill from a reservoir of a constant height. A test was devised to rule out the possibility that the reduced pressure was an artefact resulting from the use of the perfusion system. The test consisted in occluding the inflow early in diastole, preventing further filling, and making the relaxation practically isometric. This procedure enabled the ventricle to lower the pressure even more than when it filled, indicating that the ventricular muscle itself develops the reduced pressure—in other words it aspirates the fluid. Light was thrown on the mechanism by which a reduced pressure is produced by the above procedure. For it was found that the curve relating the ratio $\frac{\text{diastolic pressure}}{\text{diastolic volume}}$ at different heart sizes when relaxation occurred with no filling, coincided with the curve depicting this ratio when the same heart was allowed to fill with its diastolic pressure set. The process is therefore to be explained on the old idea of O. Frank that the relaxed heart has a smaller $\frac{\text{pressure}}{\text{volume}}$ ratio than the contracted one. Since the volume at the end of ejection is smaller than at the start the pressure will fall below the zero level of the system, the amount depending on the rapidity with which filling can occur. We can conclude that ventricular filling is as much an active process as emptying. The mechanism for both filling and emptying is dependent on some reversible physico-chemical process which alters the elasticity coefficient of the heart muscle between two definite limits, the maximum in the contracted and the minimum in the relaxed ventricle.

*The influence of varying amounts of protein on weight loss of two hogs in undernutrition.* ROBERT W. KEETON and SELMA OLSON.

Two male hogs, three years of age, weighing respectively 160 and 140 kilos were used for the experiment. They were confined to metabolism cages and fed during a preliminary period of seventeen days on an adequate ration containing 19.1 calories per kilo (basal requirements). During the experimental period (47 days) the ration contained 15.28 calories (basal minus 20 per cent). In one hog the protein of the ration was 0.94 gram per kilo (19.6 per cent of his total energy requirements) and in the other 0.036 gram per kilo (0.6 per cent of his total energy requirements). These animals lost weight uniformly and at equal rates. Even in the case of the

hog fed 0.036 gram of protein per kilo the weight loss may be ascribed largely to fat.

*The effect of a solution of gum acacia in restoring diminished body fluid.* NORMAN M. KEITH and MAURICE A. WALKER.

Water loss in dogs was produced by timed intravenous injections of 50 per cent sucrose solution, as previously described by Keith. The fluid lost by diuresis was then restored by the intravenous injection of a solution of 0.8 per cent sodium chloride, or solutions containing 0.9 per cent sodium chloride and various amounts of gum acacia. The solution of sodium chloride caused a rapid return to normal of blood volume, as indicated by the concentration of hemoglobin. However, when the estimated loss of fluid in tissues was replaced with a solution of sodium chloride and the calculated decrease in blood volume restored with a solution of 6 per cent gum acacia, there was a dilution of the hemoglobin of the blood which persisted for several hours. On the other hand, when a similar amount of solution of 6 per cent acacia was injected first, followed by the solution of sodium chloride, the concentration of hemoglobin did not return to normal for several days. Toxic manifestations were not observed if the amount of gum acacia injected did not exceed 1.2 grams for each kilogram of body weight. If the entire loss of fluid was replaced by a solution of 6 per cent acacia (4.8 to 6.1 grams of acacia for each kilogram of body weight), the blood volume was increased and urine was excreted, but toxic symptoms developed and the animals died.

In several experiments a reduction of blood volume was produced by arterial hemorrhage. The subsequent injection of a solution of 6 per cent gum acacia, in amounts equal to the blood lost caused an immediate restoration of circulating blood volume, as shown by the determinations of hemoglobin and of the hematocrit values, and also the excretion of a large volume of urine which was sometimes greater than the amount of solution of acacia injected. These results indicate that when blood volume is decreased by hemorrhage, the presence of an optimal amount of solution of gum acacia and sodium chloride in the circulating blood tends to increase the volume of blood and actually to cause true diuresis. A possible explanation of these observations is that gum acacia not only holds water in the blood, but may even withdraw it from the tissues into the blood, preparatory to the excretion of water by the kidney.

*On the possible respiratory center in the cephalic brain-stem.* ALLEN D. KELLER.

The reasons for believing that the cephalic brain-stem plays a tonic rôle in the control of respiration have been briefly reviewed in a previous paper.[1] Further evidence to support this view was likewise presented; moreover it was suggested that the slowing of respiration that results from cutting the lateral regions of the stem at an upper medullary or pons level might be due to an inhibition of bulbar centers mediated through tracts running laterally. At the time the above paper was going to press, such a possibility was strengthened by observing a vagal hyperpnea of 140 per minute in a "medullary preparation." An attempt to answer this question was made by trying to defunction the cells at various levels of the stem

[1] Keller. 1929. This Journal, lxxxix, 289.

by eliminating their blood supply. The carotids were first tied and then the basilar artery was clamped at various levels.

In some preparations clamping the basilar artery with intact vagi slowed the rate and increased the amplitude of respiration in the same manner as complete sectioning of the stem[2] at the pontal levels. Cutting the vagi after clamping was followed by a still further slowing of rate and increase in amplitude while complete respiratory failure resulted very shortly thereafter.[2,3] The same results were obtained when the vagi were cut before the clamping of the basilar artery. These findings strongly suggest that cephalic tonic respiratory cells were defunctioned because of lack of an adequate blood supply.

In other animals polypnea or ordinary respiration was unaffected by clamping the basilar artery either before or after vagi section. This fact does not eliminate the possibility that polypnea and ordinary respiration after vagi section are dependent on cephalic centers, for blood still reaches the circle of Willis through anastomoses (not complete) of the inferior and superior cerebellar arteries. This is easily demonstrated by injected specimens or, better still, by clamping the basilar artery and then cutting it cephalad to the clamp. Well oxygenated pulsating blood then escapes through the open end of the cut basilar artery.

*Types of periodic breathing observed as a result of placing lesions in the brain-stem.* ALLEN D. KELLER.

In preparations under a surgical depth of amytal anesthesia, two distinct types of periodic breathing have been observed both after complete section of the stem, and after section of the lateral regions of the stem at a pontal or upper medullary level.

One type is that of a period of respiratory arrest in the inspiratory phase followed by a period of regular respiratory movements. The inspiratory arrest is due to tonic impulses from the thoracic and abdominal wall resulting from a band tied about this region for recording purposes. Likewise, active inspiration is readily induced during expiratory arrest by applying light pressure on the skin. (Skin of thorax is most sensitive.) The inspiratory arrest ("inspiratory cramp",[4] "apneusis"[5]) is not due to inspiratory rigidity resulting from the sectioning of the rubro-spinal tracts,[6] since they can be cut without any signs of rigidity of the inspiratory muscles (cat 23).[7] Moreover, a typical decerebrate preparation shows no signs of a prolonged inspiratory phase.

The other type is that of a period of respiratory arrest in the expiratory phase followed by a period of regular respiratory movements. The expiratory arrest is of vagal origin. The inspiratory stand still is readily inhibited by touching the mucosa of the pharynx.

The periodic panting that results from placing a unilateral lesion during light anesthesia polypnea is not due to the permanent cutting out of one-half of a bilateral mechanism, since in chronic preparations with a three

[2] Markwald and Kronecker. 1880. Arch. f. anat. u. physiol., 443.
[3] Travan and Boock. 1922. Journ. Physiol., lvi, 331.
[4] Markwald and Kronecker. 1880. Arch. f. Anat. u. Physiol., 443.
[5] Lumsden. 1923. Journ. Physiol., xv, 81.
[6] Henderson and Sweet. 1929. This Journal, xci, 1.
[7] Keller. 1929. This Journal, lxxxix, 289.

quarter section of the stem typical polypnea is readily induced without any signs of periodic breathing. It likewise is not due to any temporary inhibitory effect produced by cutting the intra-stem fibers of the 5th or 9th cranial nerves, since section of these nerves before they enter the stem has no measurable effect on polypnea.[1]

*Observations on the localization of the heat regulating mechanisms in the upper medulla and pons.* ALLEN D. KELLER.

The problem of reducing the nervous mechanisms connected with temperature regulation to anatomical terms is being approached by determining the course taken by the tracts concerned as they pass through the mid-brain, pons and medulla. The factors being tested are: 1, the ability to maintain a normal rectal temperature, *a*, at an ordinary room temperature, and *b* at low extremes of temperature; 2, the presence of shivering; 3, panting, and 4, sweating.

The above mechanisms are still intact after a complete hemisection of the stem through the caudal level of the pons and likewise after a medial transverse lesion that severs completely the medial longitudinal fasciculi and reaches slightly lateralad into the medial reticular formation at a caudal pons level. A preparation with a medial transverse lesion that reaches slightly lateralad into the lateral reticular formation retains only the ability to pant and to maintain a normal rectal temperature at room temperature. A cat with a three-quarter section of the stem at a pontal or upper medullary level retains only the ability to pant. The panting mechanism is not impaired except possibly that the rectal temperature at which typical panting begins is raised. Typical panting (170 per minute, mouth open, tongue out) has been obtained in a chronic midbrain preparation (section passing through anterior end of superior colleculi dorsally and just caudal to the mammillary bodies ventrally) in which all the other above mentioned mechanisms were eliminated by the section. This demonstrates the anatomical independency of the polypnea center from that of the chief heat regulating center, assuming that the heat regulating center is located at the thalamus level in the stem.

*Some physiological variations in peripheral blood cell counts.* FANNY KENYON and ICIE G. MACY.

The range of physiological variation of leucocytes, erythrocytes, differentials, and hemoglobin over 13 consecutive hours under controlled conditions has been presented for 11 professional women, 4 of whom served as subjects for two periods. A tendency for the leucocytes to increase through the day is suggestive of rhythmical physiological variations and duplicate studies point towards a possible individual rhythm. The ingestion of a light diet was not a determinant factor in the physiological variation of the curves presented. In the differential counts the polymorphonuclear tend to follow closely the total white cell curves. The erythrocytes show a physiological variation during the day.

*A stronger coagulant for use with heparin-plasma.* JOSEPH T. KING.

The extract frequently used in tissue culture work for coagulating heparin-plasma is made by disintegrating a ten-day chick embryo, drained

[1] Conant, James S. Medical thesis. Yale University, 1930.

as free of blood as possible, in 10 cc. of Tyrode solution. It has been found that the coagulating power of the extract can be increased by the addition of small amounts of plasma (about one part plasma to 60 parts extract) which clots promptly in the extract. The slight amount of fibrin formed in this preliminary coagulation can be removed. The extract is then found to be a more powerful coagulant for heparin-plasma and to retain its power over a longer period than the original extract.

*Further evidence that dextrose is more easily used than any of the other sugars.* L. P. KNEER and W. E. BURGE.

It is generally accepted and taught that levulose is more easily used than dextrose or galactose. Contrary to this generally accepted view we have found and reported that dextrose, the sugar normally presented to the tissues, is used more easily than levulose or galactose. In this investigation the rate of utilization of maltose, sucrose and lactose was determined. This was done by introducing two goldfish, of approximately the same size and with a combined weight of about five grams, into 100 cc. of 0.1 per cent of these different sugar solutions in beakers. Sugar determinations were made according to the method of Benedict at the beginning and after 30 hours, at the end of the experiments. It was found that the fish used 63 per cent of the maltose, 45 per cent of the sucrose, and 40 per cent of the lactose. It is assumed that the maltose was used more rapidly than the lactose or sucrose because maltose yielded two molecules of dextrose on digestion which is easily used while the other two sugars only yield one molecule of dextrose and one of levulose and one of galactose respectively which are more difficult to utilize than the dextrose.

The fish were supplied with oxygen by bubbling air through the solutions in the beakers. Bacterial action was eliminated by the use of ultra violet radiation.

*Visceral reactions associated with induced nystagmus.* JAMES E. LEBENSOHN.

The study was undertaken to determine the nature of visceral reactions that accompany clinically induced optical and labyrinthine nystagmus. The state of gastric motility was followed by kymographic tracings from a swallowed balloon, and by fluoroscopic observations. Increased tone of the stomach occurs during the period of the induction to return to normal or below on cessation of the application of the stimulus even in spite of the persistence of the induced vertigo.

*Calorigenic effects of anterior lobe pituitary hormones.* MILTON O. LEE.

The basal respiratory metabolism was found to be depressed in giant rats produced by the daily injection of extracts containing the growth promoting principle of the anterior lobe. In four such rats the average basal metabolic rate was 12 per cent lower than in their controls. Three to four weeks after the administration of the extracts was stopped the metabolic rate rose nearly to the level in the controls. The increase in size in itself does not seem to be responsible for the decrease in metabolism, since such low metabolic rates are not found in normal rats of nearly the same weight.

In adult rats of normal weight the administration of extracts containing the growth promoting principle causes in most cases a decrease in the basal metabolic rate. In some rats this decrease has amounted to as much as 32 per cent, and has continued for as long as three weeks after the use of the

extract was stopped. In some rats there is apparently no effect on the respiratory metabolism. Increases have not been found in any rats, except for a period of a few hours after giving a dose of the extract. This increase is probably due to the calorigenic effect of the protein in the extract since boiled extracts have the same effect. Extracts containing the sex stimulating principle have shown little or no effect on the basal respiratory metabolism. Commercial anterior lobe preparations given by mouth have been found to be entirely without effect on the metabolism.

*Relation of the autonomic system to the inorganic constituents of blood.* E. G. LIPOW and W. K. WEAVER.

First were established the effects of ether anesthesia on the plasma content of calcium, phosphorus, potassium and magnesium. With few exceptions out of 20 experiments, calcemia decreased progressively during two hours; inorganic phosphorus showed an increase of more than 50 per cent except in two experiments; sodium showed an initial fall with a terminal rise; potassium values fluctuated considerably but the predominant effect was a progressive decrease; magnesium concentrations fluctuated so much that no predominant effect could be established. A composite curve showed a fall followed by a terminal rise above normal. In another series, both intact vagi were stimulated for a half hour with a tetanizing current so weak as not to interfere with respiration but which slowed the heart about 10 per cent. Determinations made over a period of two hours from the beginning of stimulation did not alter the inorganic phosphorus curve, produced a progressive hypercalcemia; caused a rise of sodium concentration followed by a fall below normal; produced a progressive increase in potassium and a fall in magnesium. In a third series, both vagi were severed in the neck and stimulated as above. The results were: a still greater hypercalcemia; a delayed hyperphosphemia of less magnitude than in the other two series; decrease in sodium of greater magnitude than in the first series followed by a return to normal; potassium increased to some extent but tended to return to normal; there was a very great progressive increase in magnesium.

*The regulation of the respiration in nephritis with nitrogen retention.* ROBERT O. LOEBEL, HAROLD E. HIMWICH and DAVID P. BARR.

It would be very satisfactory if, in health and disease, the rhythm, the rate, and depth of the respiration could be shown to be determined in the last analysis by one physiological factor. For that reason the rôle of oxygen and of carbon dioxide has received very great attention. The experiments to be reported in this paper are studies of these functions in patients suffering from nephritis which profoundly influences these blood gases. The blood was obtained by puncture of the brachial artery. Oxygen content and capacity, and three-point dissociation curves were then obtained from determinations carried out in the constant pressure Van Slyke apparatus; respiratory data by employing an army mask and the Bailey spirometer. Several precautions were employed. Cases primarily cardiac and those showing periodic breathing were excluded; those receiving sodium bicarbonate were omitted, since that drug might easily affect the acid base balance of the blood; those receiving morphin were excluded, since that drug might diminish the sensitivity of the respiratory center and mask some other primary influence.

In all, eleven observations are reported on nine cases of chronic nephritis and six on three cases of acute anuria.

1. Eight out of nine cases of advanced chronic nephritis with nitrogen retention, which were comparatively uncomplicated, showed over-breathing and definite acidosis of the arterial blood. In a single exceptional case the clinical picture was extremely complicated, and over-breathing was associated, on one occasion, with a normal hydrogen ion concentration, and on another with alkalosis.

2. In two out of three cases of mercury bichloride poisoning with anuria, there was no stimulation of respiration in the presence of marked acidosis.

3. There is no apparent relation between anemia and over-ventilation in the chronic or acute cases studied.

4. The equations for the buffer value of normal blood derived theoretically by Van Slyke, Wu and McLean are shown by experiment to apply to the blood of these nephritic patients.

*Relation of stainability and electric potential differences to the pH value.* Jos. Lozner.

In tissue—as well as in certain artificial systems of water-immiscible substances—basophilic staining is associated with a positive potential, acidophilic staining with a negative potential. The same is true for a system containing gelatin on the alkaline side and on the acid side of the iso-electric point, according to Loeb.

Whether the concomitant changes of stainability and e.m.f. are brought about in tissues chiefly by variations of its water-insoluble constituents or its water-soluble constituents can not yet be decided. Even if water-soluble constituents, or pH changes play the most important rôle, this would not prove that proteins play a predominant rôle in the production of bio-electricity because the electromotive effect of Loeb's gelatin cell can also be produced by means of fats or other water-immiscible fluids.

To prove this a mixture of equivalent parts of a fatty acid and an oil-soluble amine in a neutral fat was shaken with aqueous buffer solutions of varying pH. This exhibited the same type of stainability as gelatin, namely: staining acidophilic below a certain pH value; and basophilic above a certain pH value.

We also find—just as in the case of Loeb's gelatin cell—that the acidophilic mixture has a negative potential relative to the basophilic which is positive, as demonstrated in the following diagram:

| − | low pH acidophilic | fatty or oily mixture | high pH basophilic | + |
|---|---|---|---|---|

*The relation of duodenal motility to the resistance of bile flow into the duodenum.* H. C. Lueth.

One of a series of studies undertaken to study the physiology of the sphincter of Oddi was to ascertain if a relation existed between duodenal motility and the inflow of common duct fluid into the lumen of the duodenum. The apparatus used was essentially that described by Elman and McMaster[1] which involved the determination of the amount of pressure necessary to cause common duct fluid to pass into the duodenum, the "in-

[1] Elman and McMaster. Journ. Exper. Med., 1926, xliv, 151.

flow drops" being recorded and the duodenum intact. We added to this procedure a balloon record. A small balloon, two inches long with a small rubber tube was inserted into the stomach just proximal to the pylorus and passed into the duodenum so that the balloon was in that portion of the duodenum in which the intramural portion of the common duct is located, the balloon being held in place by a suture at the site of incision in the stomach. Dogs under morphine-ether or barbital ether anesthesia were used. The pressure level in the common duct was raised to the point that some inflow was recorded and then held constant at this level. It was found in those dogs which manifested active duodenal motility that the inflow was intermittent and synchronized with the duodenal motility. This explains the fluctuating resistance to the flow of bile into the duodenum observed by Elman and McMaster, and proves, we believe, that the duodenal musculature plays a predominant rôle in the resistance offered to the flow of bile into the duodenum.

*The effect of adrenaline on the auricle of elasmobranch fishes.* BRENTON R. LUTZ.

Adrenalin chloride, 1 in 50,000 to 1 in 25,000 produced slowing or temporary complete inhibition of the beat of an isolated sinus-auricle preparation made from the heart of each of the following fishes, Raia erinacea, R. diaphanes, R. scabrata, and Squalus acanthias. The amplitude of beat also decreased, but both rate and amplitude recovered, the latter frequently becoming temporarily greater than the original. Chloretone in quantities equivalent to the amount present in adrenalin chloride of the concentrations used to produce typical effects was ineffective. The inhibitory effect of adrenaline on the elasmobranch auricle is interpreted as the response of an unbalanced parasympathetic mechanism in a heart which lacks a sympathetic accelerator innervation. Since any unusual sensory stimulation of the gills, skin, or viscera evokes reflex inhibition of the heart and sometimes of respiration, it is suggested that the vago-mimetic effect of adrenaline on the heart is also an emergency response.

*The innervation of the heart of the elasmobranch, Scyllium canicula.* BRENTON R. LUTZ.

Morphological and physiological observations showed that two branches of the vagus go to the heart of Scyllium canicula, each carrying afferent and efferent fibers. One branch takes its origin from the post-branchial ramus of the fourth branchial division (fifth branchial nerve), and the other arises from the main visceral trunk of the vagus. Reflex cardio-inhibition sometimes accompanied by reflex respiratory movements can be obtained in a fish with all nerves cut, except the vagus supply to the gills and either cardiac ramus, on mechanical or faradic stimulation of the ventricle.

Under experimental conditions there is vagal tone, since cutting the vagus on one side gave a considerable increase in heart rate which was further augmented when the remaining vagus was cut.

No acceleration of the rate of the heart occurred in fishes with the gills perfused on faradic stimulation of the medulla, spinal cord, or first large sympathetic ganglion, with the vagi cut.

No slowing of the rate of the heart occurred, due to lowering of tone in an accelerator center, on reflex excitation of the cardio-inhibitory center, with the vagi cut. Both morphological and physiological evidence indicate the lack of accelerator fibers to the heart of the elasmobranch.

*Respiratory rhythm in the elasmobranch, Scyllium canicula.* BRENTON R. LUTZ.

The respiratory and cardiac responses to stopping and starting the water supply to the gills, to increasing and decreasing the rate of flow, and to complete interruption of the blood supply to the medulla of Scyllium canicula were recorded graphically. Sudden stopping and starting or increasing and decreasing the water supply evoked brief respiratory inhibition and sometimes ejection reflexes.

An increase in the rate of flow of sea water to the gills caused an increase in respiration and heart rates. A decrease caused the reverse. Too great an increase, however, evoked irregular inhibition. Inasmuch as accelerator nerves have not been demonstrated in elasmobranchs, the increase in heart rate accompanying increased respiration is probably due to inhibition of vagal tone.

Stopping the flow of water to the gills evoked brief inhibition of respiration followed by a regular rhythm at first, which sometimes became periodic and finally was interrupted by generalized muscular activity. The medullary centers remained reflexly functional for over three and one-half hours.

Interruption of the blood supply to the medulla, when there was no water supply to the gills, was followed by regular respiration at first, then periodicity and dyspnea. The medullary centers remained reflexly functional for over sixty minutes without a blood supply. It is concluded that the respiratory center is autonomous, but may be influenced by the gas content of the blood and by peripheral stimulation.

*Zoöpharmacological and phytopharmacological studies of human spinal fluid.* DAVID I. MACHT.

The author studied the effects of human spinal fluids, obtained from hospital laboratories, on living animal and plant test objects. Zoöpharmacological experiments were made on blood pressure and on isolated smooth muscle, more particularly, of the uterus. It was found that the pressure was slightly raised by injections of the various specimens and uterine muscle was stimulated to contraction by the same preparations, thus confirming the results observed by previous investigators with spinal fluid from animals and speaking in favor of its being closely related to the hypophysis. The most interesting observation, however, was the fact that specimens obtained from different persons gave quantitatively different results. Spinal fluids from cases of syphilis were no different from normal human spinal fluids in this respect.

Phytopharmacological tests were made on living seedlings of Lupinus albus according to methods developed by the writer in connection with studies on pernicious anemia, etc. It was found that while normal human spinal fluids were but little toxic for the growth of the seedlings when employed in concentrations of two per cent, the spinal fluids from cases of pernicious anemia were markedly toxic for the plants, thus pointing to the presence of pernicious anemia toxin in that body fluid. These findings are of obvious interest in connection with the problem of etiology of the spinal manifestations so commonly met with in pernicious anemia patients and may be developed into a useful method for differential diagnosis of doubtful cases.

*A new demonstration of physiological activity of corpus luteum.* DAVID I. MACHT.

It has been pointed out by Siddall and others that the weight of the uterus in the white mouse bears a definite ratio to the weight of the adult, non-pregnant animal, this ratio being over 400. Franck and other investigators have found that when white mice are given injections of the follicular hormone, the uterus is hypertrophied, and Siddall established that in such cases the ratio of the weights of the uterus and the whole animal is much below the normal ratio 400. In the present investigation, the writer attempted to determine whether the corpus luteum hormone would counteract the effects of follicular hormone on the size and weight of the uterus. Three sets of experiments were carried on simultaneously. In the first set, mice were fed a normal diet without the administration of ovarian extracts. In the second set, mice were given the same food but received a number of injections of the follicular hormone. In the third set, mice were injected simultaneously with the follicular hormone and corpus luteum extracts. After various periods of time, ranging from ten days to two weeks and more, the animals were killed, the uteri dissected, and the relation of the uterine weight to the weight of the whole animal was determined. In the normal controls, this ratio was never below 400 and was usually higher than that figure. In the mice which received injections of follicular extracts alone, a definite hypertrophy of the uteri was noted in most cases, and the ratio of the uterus to the body weight was much below 400. In the third set of experiments, the mice which received simultaneous injections of the follicular and corpus luteum extracts showed no hypertrophy of the uteri and gave the same ratio of the weights as the normal controls. Such triple sets of experiments were performed many times, always giving the same results. It therefore appears that injections of corpus luteum extracts counteract the hypertrophy-producing effects of follicular extracts. Feeding experiments with the ovarian products were also conducted and gave similar results, although a much longer period of time was necessary.

*The relation of the liver to the utilization of levulose.* FRANK C. MANN and JESSE L. BOLLMAN.

The symptoms of hypoglycemia which follow complete removal of the liver are promptly dispelled by administration of glucose. Levulose, however, is ineffective if administered a few minutes before death from hypoglycemia. If levulose is given before symptoms of hypoglycemia develop following removal of the liver the symptoms may be averted and the life of the animal prolonged. More levulose is required to prevent symptoms of hypoglycemia than is necessary when glucose is administered. A study of the rate of disappearance of injected levulose from the blood of normal and hepatectomized animals showed only a slight delay in the liverless animal. The content of glucose in the blood increased slightly following the administration of levulose in the normal animal, and after hepatectomy this increased content of glucose in the blood was more noticeable because of the lowered content of glucose before levulose was given. Symptoms of hypoglycemia, when allowed to develop, were independent of the content of levulose in the blood but were definitely related to the glucose of the blood.

It would appear that the beneficial effects of levulose on the liverless

animal depend on the conversion of levulose to glucose. Muscle glycogen can be formed in the absence of the liver from levulose administered intravenously in the dehepatized animal. We have no direct evidence whether this formation of glycogen is directly from levulose with subsequent liberation of glucose from glycogen, or whether it is dependent on the preliminary conversion of levulose to glucose, with subsequent formation of glycogen from glucose. Following the removal of the gastro-intestinal tract, pancreas, and liver, hypoglycemia develops and may be entirely prevented by the administration of glucose. The administration of levulose to these animals is entirely without effect on the content of glucose in the blood, even if it is administered for several hours prior to the development of symptoms of hypoglycemia.

*Factors affecting the distribution of lactates between blood and muscles.* E. G. Martin, J. Field, II, and V. E. Hall.

In dogs, anesthetized with amytal, continuous records of arterial blood-pressure and oxygen consumption were made. Rectal temperatures were determined at intervals throughout each experiment. Samples of carotid arterial blood, of brachial vein blood and of femoral vein blood were drawn, all within a period of six minutes. As rapidly as possible forelimb flexors (superficial and deep) and rectus femoris, gracilis and gastrocnemius-soleus muscles from hind limbs (on the side from which venous blood was drawn) were frozen in situ with carbon dioxide snow, excised, and ground to powder in liquid air. Blood lactates and muscle lactates were then determined. The experiments fall into three groups as follows: 1, venous blood from a limb contains more lactates than arterial blood, indicating that the muscles of that limb are giving off lactates; 2, venous blood from a limb contains less lactates than arterial blood, indicating that the muscles of that limb are taking up lactates; 3, venous and arterial bloods have similar lactate content, indicating that the muscles are neither contributing nor withdrawing lactates. The accompanying table gives the average data for the three groups.

| | ARTERIAL PRESSURE | | OXYGEN CONSUMPTION PER KILO PER MINUTE | LACTATES | | |
|---|---|---|---|---|---|---|
| | Early in experiment | At time of excising muscle | | Artery | Vein | Muscle |
| | | | | *mgm. per cent* | *mgm. per cent* | *mgm. per cent* |
| Group 1 (12 expts.) | 109 | 96 | 5.4 | 21 | 31.5 | 49.0 |
| Group 2 (10 expts.) | 81 | 80 | 5.5 | 27 | 19.6 | 35.6 |
| Group 3 (7 expts.) | 103 | 102 | 5.0 | 20 | 20.5 | 30.5 |

Contrary to expectation the arterial pressure averages considerably higher in the group in which the muscles are giving off lactates than in the group in which the muscles are taking up lactates. In agreement with expectation the muscle lactates are higher in the group in which lactates are being given off than in the other groups, but contrary to expectation the muscle lactates are lower in the group in which the muscles are not

affecting blood-lactate content than in the group in which the muscles are taking up lactates from the blood. No significant differences in oxygen consumption are apparent in the three groups.

*Comparative sensitivity to oxygen-want and to sodium lactate of the hearts of normal and thyroxinized animals.* DONALD MCEACHERN and E. COWLES ANDRUS.

The auricles of two rabbits were suspended side by side in a Dale bath. To one animal thyroxin had been administered in graduated doses over a period of two weeks.

The authors have confirmed the observation of J. K. Lewis, that the spontaneous rhythm of the thyroxinized heart persists after isolation at a rate conspicuously more rapid than that of the normal. They have further shown:

1. That the auricles of the thyroxinized animal are far more sensitive to the withdrawal of oxygen than the normal.
2. That their rate and amplitude are depressed by doses of sodium lactate which produce little or no effect upon the normal auricles, and
3. That recovery from the above takes place more rapidly in the normal than in the thyroxinized auricles.

The authors regard these results as indirect evidence of an accumulation of lactic acid in the heart of the thyroxinized rabbit.

*The relation of body temperature to environmental factors.* B. MCGLONE.

Recent work on the relation of body-temperature to environmental conditions raises the question of the degree of dependence of the normal diurnal variations of body temperature upon those in the environment. Observations of rectal temperatures have been made in an environment in which the dry bulb temperature has been controlled and the humidity and air-movement have been fairly uniform. The subjects did not take food and usually remained recumbent; they were clothed in cotton pyjamas. The degree of activity varied from deep sleep to slight body movements and mental activity (reading and conversation). In one experiment the degree of activity was slightly increased.

When the accustomed routine was not varied, the usual diurnal rhythm obtained even with a narrow effective temperature (basic scale) range, 0.9 to 2.0 degrees (average absolute value 23 degrees).

During sleep, both at night and day, the body temperature fell; a rapid rise precedes or accompanies waking. It is possible to reverse the nocturnal phase of the normal diurnal rhythm if the heat production due to activity is not overbalanced by a change in environmental factors. The temperature of one subject, awake at night and acting as observer, necessitating not only increased mental activity but occasional muscular activity, rose during the night, attaining a maximum at 9 a.m., and then fell to a minimum at 4 p.m. after which an increase occurred. In a second subject, awake and reading, the nocturnal fall was delayed until "beginning to feel sleepy" at 3 a.m. and persisted until 11 a.m. when on awakening the characteristic rise followed. However, one subject awake and quietly resting at night had a normal rhythm with a minimum at 3 a.m.; the dry bulb and effective temperatures were constant, 28°C. and 22.5° respectively.

*Formation of methemoglobin from rabbit's blood.* WALTER J. MEEK.

Many workers have now observed that rabbits are very resistant to the formation of methemoglobin by members of the aromatic series. So far as we know no suggestions have been made to explain this phenomenon. Since in dog's blood para amino phenol acts much more rapidly than phenetidine or aniline it was suggested to us that in the rabbit some organ might be preventing the change of aniline or phenetidine to this substance. Rabbits were therefore injected with aniline and various organs tied off on the chance that methemoglobin might then appear. In this way it was shown that the failure to produce methemoglobin does not depend directly on the kidney, intestines or liver. Macerated liver does however delay methemoglobin formation in vitro which is in accordance with Neill's finding that liver tissue has considerable capacity in reducing methemoglobin to hemoglobin. In vitro many of the phenols will form methemoglobin from rabbit's blood although the rate is much slower than it is for dog's blood. It has been found that potassium ferricyanide and quinone form methemoglobin as readily in rabbit's as dog's blood. The difference then does not seem to depend on any difficulty in oxidizing the rabbit hemoglobin. On the other hand aniline, para amino phenol, acetanilid, hydroquinon and phenetidine, which must themselves be hydrolized, reduced or oxidized, act less readily in rabbit's blood. It would seem then that something about rabbit's blood interferes with these oxidations, reductions and hydrolyses.

*Studies of the response to calcium medication in the hypoglycemia of carbon tetrachloride poisoning.* A. S. MINOT and J. T. CUTLER.

The protective and curative effect of calcium salts in carbon tetrachloride poisoning has already been reported.[1,2] These earlier studies also showed that in carbon tetrachloride poisoning there is an increase in the concentration of guanidine or guanidine-like substances in the blood and that this increase is followed, in dogs unprotected by calcium, by an extreme hypoglycemia. The appearance of dangerously low blood sugar levels can be prevented by adding calcium salts to the diet or if hypoglycemia is allowed to develop it can usually be relieved by intravenous calcium medication. The elevation of the blood sugar after calcium is so prompt as to resemble the hyperglycemic response to adrenalin.

The necessity for adequate calcium for normal sympathetic response to adrenalin has been repeatedly demonstrated by various investigators. Experiments have recently been carried out to see whether any evidence could be obtained indicating that the sympathetic system was involved in the response to calcium medication. Our work thus far has brought out the following points:

1. Adrenalin alone fails to give any restoration of blood sugar or relief of symptoms.

2. Intravenous calcium medication whether in the form of calcium lactate, chloride, or gluconate gives a marked improvement in the general condition of the poisoned animal and a very prompt elevation of blood sugar. This amelioration of symptoms and increase in blood sugar persists for an hour or two when further calcium must be administered if the animal is to be saved.

[1] Minot, A. S. Proc. Soc. Exper. Biol. and Med., 1927, xxiv, 617.
[2] Minot, A. S. and J. T. Cutler. Journ. Clin. Invest., vi, no. 3, December 1928.

3. Soon after calcium has been administered adrenalin gives a typical transient hyperglycemic response over and above that already caused by the calcium.

4. Calcium medication does not affect the guanidine level in the blood.

5. Ergotamine tartrate in doses sufficient to paralyze the sympathetic endings, given previous to calcium medication prevents the hyperglycemic response to calcium.

6. Ergotamine tartrate given to animals with increased guanidine concentrations in the blood, but protected by calcium against development of hypoglycemia, will promptly cause a marked fall in blood sugar.

We believe that these findings indicate that the effect of calcium on the hypoglycemia of carbon tetrachloride poisoning depends upon sympathetic activity.

*On the relation between the shape of stimulus and the shape of the nerve action potential.* A. M. MONNIER.

A mathematical theory of stimulation has been worked out based on the following hypothesis: In a nerve stimulated electrically a phenomenon takes place with rises and falls according to a double exponential formula. Stimulation occurs when this phenomenon has reached a definite magnitude. All phenomena of electrical stimulation are well accounted for on the basis of this theory: stimulation by alternating currents, direct currents, rising currents, condenser discharges, opening of direct current and summation, etc.

This theory gives also a good illustration of Lapicque's selective stimulation by double exponential waves (discharges of a double condenser circuit) and allows one to calculate the shape of the hypothetical phenomenon in nerve, on the basis of the shape of the double exponential wave used as stimulus. A study of stimulation of nerve by double exponential waves has been started in the Sorbonne and completed in St. Louis. Observation of very small threshold action potentials with Gasser and Erlanger's cathode ray oscillograph,[1] has permitted great accuracy in the determination of the relation between the magnitude of the stimulating double exponential wave and its duration. The curves obtained verify the mathematical theory and so give the shape of the hypothetical phenomenon. The shape is characterized by a ratio of the two exponentials close to 1. Experiments on nerve have also demonstrated that such a ratio characterizes the shape of the double exponential wave which has the greatest stimulating effect.

By a simple electrical circuit one may accurately reproduce the wave form derived mathematically to give an image of nerve action potentials. Cathode ray oscillograms obtained from such a circuit, when using double exponential waves characterized by a ratio close to 1, simulate the nerve action potential form as obtained by Erlanger, Gasser and Bishop.[2]

*The effect of glycine and alanine upon the oxygen consumption of the heart-lung preparation.* A. G. MULDER.

The oxygen consumption of the Starling heart-lung preparation was measured both before and after the injection of glycine and alanine. Dogs starved for about eighteen hours were used. The ventricular volume and

[1] Gasser, H. S. and Joseph Erlanger. 1922. This Journal, lxii, 496.

[2] Erlanger, J., H. S. Gasser and G. H. Bishop. 1924. This Journal, lxx, 624.

blood pressure were recorded and in some instances the heart rate and blood flow, oxygen consumption was measured, by the method of Starling and Visscher, for ten minute intervals several times before and after the injection of the amino acid.

Nine experiments were performed. The introduction of the amino acids dissolved in blood caused a decrease in ventricular volume which later tended to increase and exceed the volume registered before the injection. As a rule the oxygen consumption fell with the ventricular volume and rose with the ventricular volume. In some experiments variations to this occurred and after the injection of the glycine there was no exact correspondence between ventricular volume and oxygen consumption. Adrenalin was sometimes injected at the end of an experiment as a check to test the ability of the preparation to respond to a calorigenic stimulus. Adrenalin always much increased the oxygen consumption.

The data, we believe, tend to show no increase in oxygen consumption, due to glycine and alanine, other than that which can be explained, in most instances, by the rise in ventricular volume.

The data for one of the experiments are as follows:

| TIME | $O_2$ USED IN 10 MINUTES | VENTRICULAR VOLUME | BLOOD PRESSURE | | HEART RATE | BLOOD FLOW |
|---|---|---|---|---|---|---|
| | | | Systolic | Diastolic | | |
| *minutes* | *cc.* | | | | | *cc. per minute* |
| 0 | 65 | 0 | 156 | 146 | 160 | 217 |
| 12 | 66 | +1 | 154 | 144 | | 208 |
| 24 | 71 | +2 | 154 | 144 | 150 | 208 |
| 36 | 5 gm. of alanine introduced | | | | | |
| 46 | 67 | 0 | 158 | 142 | 140 | 217 |
| 58 | 75 | +6 | 158 | 139 | | 208 |
| 70 | 84 | +14 | 158 | 136 | 160 | 200 |
| | 1 cc. of adrenalin introduced | | | | | |
| 82 | 145 | | | | | |

*The gastric hunger mechanism. II. The effect of diet.* MICHAEL G. MULINOS.

Dogs in insulin shock show an increase in gastric motility and tonus. Glucose, administered by stomach or parenterally in large amounts, depresses both the activity and the tonus of the stomach. Soon after the exhibition of the glucose (3 to 30 minutes), there is a return of activity to the pre-insulin level, a phenomenon which suggests that the glucose does not depress the spontaneous activity of the normal stomach.

For periods of from 3 to 10 months, 8 dogs were fed on a diet of lean boiled meat and bread, in the proportion of 1:2. Although they ate all of the food offered, eventually they fail to gain, or may actually lose, in weight. Those kept on this diet for long periods may lose their appetites; they catch "colds" very easily; and it is difficult to keep them free from mange. Gastric motility remains normal, although the quiescent periods become longer and more frequent.

*The response to insulin is gradually lost,* only a slight tonus undulation being produced by large doses of the hormone. This is removed by glucose. When a cake of yeast is added to the daily ration, the appetite improves, the dogs gain in weight and activity. *Yeast brings back the lost gastric response to insulin hypoglucemia.*

During the experiments, it was found that the dogs became resistant to insulin, and it was necessary to increase the dose severalfold, in order to induce shock.

After the addition of the yeast, the effective dose of insulin gradually returned to or even below the previous level. The tolerance of dog III increased from 0.3 unit to 2.0 units per kilo, during a period of 9 months, then decreased to 0.25 unit per kilo, on the fourth day following the addition of yeast. At all stages of the experiment, the *intravenous administration of insulin causes a depression of gastric activity and tone*, lasting from a few minutes to half an hour. This is not due to the 0.1 per cent phenol used as preservative.

During the 2 years that the animals were kept on this diet, the following factors as causative of the change of gastric response to insulin were eliminated: a, oestrus—all the animals were females; b, seasonal changes; c, salt deficiency—by the addition of an adequate salt mixture; d, source of insulin, by using that of two different manufacturers.

One dog had a peculiar gastric reaction to insulin. The normal gastric motility was depressed, and the tonus fell to below the base-line, as soon as shock set in. There was such extreme dilatation of the stomach, during this time, that even 150 cc. of air in the balloon failed to raise the tonus to the original control level. (Commonly, 10 to 15 cc. of air are enough.) This peculiar effect disappeared on the meat-bread diet, to return again when yeast was added. In all cases, canned vegetable soup or tomatoes had the same effect as the yeast.

*Conclusions:* a. Yeast increases the susceptibility of dogs to insulin. b. Insulin hypoglucemic gastric hyperactivity is dependent upon a diet having an adequate amount of a yeast factor, probably vitamine-B. c. Lack of this factor increases the tolerance of dogs to convulsive doses of insulin.

*Conditions for metabolism of carbohydrate in aseptically incubated liver brei.* JOHN R. MURLIN.

Rabbit's liver ground in a sterile press and incubated in saline solution under aseptic precautions presents many points of interest as regards the metabolism of the contained carbohydrate. Diastase already present or added acts best in the absence of aeration. Lactic acid added hastens glycogenolysis up to a certain concentration, beyond which it retards. With insulin lactic acid causes more rapid disappearance of true sugar. The lactic acid added is all recovered.

*The retention of sugar by the skin.* JOHN W. PALMER.

In confirmation of the observations of Folin, Trimble, and Newman[1] intravenously injected glucose is temporarily stored in the skin; the normal fasting level is restored at the end of five hours. Heat treated glucose was not stored in the skin in as large amounts and the fasting level was restored in about two-thirds the time required for glucose to disappear. Injected fructose behaved like the heat-treated glucose, a fact connected with the more rapid oxidation of fructose in the animal body. Heat treated fructose accumulated in still smaller amounts but with no reduction in the time of its removal.

[1] Journ. Biol. Chem., 1927, lxxv, 263.

*The physiology of the gastric motor mechanism in the crab.* T. L. PATTERSON.

Comparative studies in the physiology of the gastric motor mechanism has been extended to the crustacea. The large crab (Cancer productus) of the Pacific coast was employed and the investigation was conducted at the new Jacques Loeb Laboratory of the Hopkins Marine Station of Stanford University. During the experimentation the animals were kept in a large vivarium provided with running sea-water which was aerated. The balloon was introduced via mouth by pushing back the valvular fold covering the opening into the esophagus at the base of the mandibles after the chela had been wedged. Later it was found advisable to amputate the endognathal palps arising from the meropodites of the third pair of maxillipeds, since the animal usually learns to use these appendages to advantage in working the balloon out of the stomach. The contractions of the gastric mill are periodic, the periods increasing in activity and length until in extreme fasting they become almost continuous on an elevated tonus. In the earlier stages of fasting there is a marked increase in the gastric tonus during the period of activity which terminates in a fall. The individual contractions of the gastric mill are rapid with practically no intervening periods of rest, while the small and rapid oscillations seen in the curves are due to the action of the balers which are the terminal appendages of the exopodites of the various pairs of mouth parts by whose vibratory motion water is kept flowing over the gills. The introduction of small quantities of weak alkali or acid into the water near the animal as well as body movements produces temporary inhibition of the gastric contractions. Isolation of the stomach from the cerebral ganglion (brain) by sectioning the circum esophageal commissures is without effect, thus demonstrating that the stomach of this animal is independent of the higher nervous centers for its activity.

*The deportment of cats and their susceptibility to experimentally produced convulsions after median longitudinal incision of the midbrain or medulla.* F. H. PIKE, W. S. MCCULLOCH and M. N. CHAPPELL.

Following out a series of experiments on the elimination of the rubrospinal tract, by median longitudinal incision of the mid-brain, (Arch. Neurol. and Psychiat., 1930, in press) at the level of the decussations of Forel and Meynert, in which tonic convulsions under absinthe were either absent or appeared only after excessive doses, we have extended this procedure to section of the decussation of the middle cerebellar peduncle (brachium pontis) and the decussation of the lemniscus. Clonic convulsions are elicitable in such animals within a few hours after the operation but the minimal convulsive dose of absinthe is larger than ordinary. Tonic convulsions appear as usual when lethal doses are administered.

An animal with a median longitudinal incision of the decussation of the lemniscus showed a curiously ataxic gait differing somewhat from that seen after ordinary cerebellar lesions. It would walk about, swaying to one side or the other, but would only occasionally fall over. At times it would jump straight into the air and come down on all four stiffly outstretched legs. We have not seen this deportment after any other experimental lesion of the central nervous system. It would frequently rear and fall over backwards, but there were no wild scrambles such as follow this manoeuvre in a cat with a cerebellar lesion. Median longitudinal incision of the pyramidal decussation does not give rise to this deportment (Amer. Journ. Psychiat., September, 1929).

*Unilateral extirpation of the vestibule following median longitudinal incision of the midbrain in cats.* F. H. PIKE and PAGE NORTHINGTON.

In two animals which had survived median longitudinal incision of the midbrain for some months, the incision had gone slightly to one side of the median line so that the vertex of the head of each animal was to the right and the chin to the left. In one, a small lesion of the left motor cortex had been made about a month after the midbrain incision.

In the first animal, with median incision of the midbrain only, the left internal ear was removed. Immediately after the operation, typical vestibular nystagmus of the usual type appeared and the vertex turned to the left, as is usually the case. The nystagmus did not appear to be as violent as usual and disappeared in about three days. In the other animal the right internal ear was removed. The torsion of the head to the right, previously existing, was increased so that the chin pointed up into the air and to the left. If one stood to the right of the cat, and called it, when its head was turned with the vertex far to the right and the left ear almost vertical, the cat invariably turned to the left in answering the call.

*The combined action of adrenalin or caffeine and absinthe in experimentally produced convulsions.* F. H. PIKE and JOHN NOTKIN.

We have previously shown[1] that certain substances such as lactic acid and acid fuchsin, when used in conjunction with absinthe, when the dose of each of the constituents is less than that necessary to elicit a convulsion if used alone, will produce convulsions. Neither caffeine nor adrenalin alone, if used in any ordinary concentration, will elicit convulsions when injected intravenously. We have found that, in cats, the usual minimal convulsive dose of our standard solution of absinthe, when used alone, is 0.035 cc. to 0.04 cc. per pound of body weight. Half this dosage, or less, is all that is necessary to elicit convulsions when injected along with, or shortly after, one or two grains of caffeine or an undetermined but not excessive dose of adrenalin (commercial preparation). The convulsions usually last longer when either of these substances is used along with the absinthe than when the absinthe alone is used. Adrenalin and absinthe are not apt to be lethal unless the dose of absinthe becomes considerable.

Caffeine and absinthe are usually lethal when combined. In one typical experiment, a first injection of two grains of caffeine was followed at intervals of ten to fifteen minutes by three successive injections of one grain each. Fifteen minutes after the last injection of caffeine, 0.01 cc. per pound of body weight of absinthe was injected. Excitement but no convulsions followed. Ten minutes later a second dose of absinthe, 0.015 cc. per pound of body weight, was administered. Clonic convulsions appeared immediately. They lasted about five minutes, after which no more respiratory movements were seen. Five minutes later, rigor mortis was plainly noticeable in the muscles of the limbs, and fifteen minutes later it was fully established. The brain showed venous congestion on gross examination.

*Further effects of methyl guanidine upon autonomic nerves.* WILBUR F. POTTER.

In addition to the effects of methyl guanidine sulphate on the vagus

[1] Osnato, M., F. H. Pike and J. Notkin. Arch. Neurol. and Psychiat., 1930, in press.

nerve and the secretory fibers of the chorda tympani nerve of the dog under ether anesthesia which have been reported previously, the action of this compound upon other tissues innervated by autonomic nerves has been investigated. It has been found that its effect upon blood flow through the submaxillary gland is not profound or constant provided the blood pressure be kept uniform. When the blood pressure is maintained at a constant level sometimes a slowing of blood flow through the gland results, at other times an acceleration of blood flow occurs. If the blood pressure is not controlled a very marked increase in the blood flow from the gland is invariably obtained. As regards the secretion of pancreatic juice, not only does this substance fail to cause a secretion but if a secretion has already been stimulated by an injection of 0.4 per cent HCl into the duodenum this secretion is markedly slowed if not stopped by the subsequent injection of methyl guanidine into the femoral vein. On the bronchioles the substance produces a rather marked dilatation that cannot usually be increased by atropine or adrenalin injections. Massive doses will completely block the chorda tympani to electrical stimulation. After the block has been obtained it is quite persistent and is not removed by injections of calcium salts. An investigation of methyl guanidine effects upon the small intestine and the urinary bladder is now in progress.

*The jurisdiction of the all-or-none law.* FREDERICK H. PRATT.

Complete discharge independent of size of stimulus, such as is characteristic of vertebrate nerve, heart, and skeletal muscle fiber, must be challenged as a basic property of conducting tissue (Fischl and Kahn, H. J. Jordan, W. Burridge, S. Gelfan). The latter's demonstration of submaximal twitches in the muscle fiber has been conclusively verified. Results, although compelling a fundamental restatement of the all-or-none law, simplify the problem of response by removing an apparently artificial boundary.

Grading can be accomplished, sometimes with ease, sometimes with great difficulty, by direct stimulation of the well-isolated fibers of the retrolingual membrane or vocal sac by electrodes not necessarily very fine, or even in contact with the fiber. The responses are twitches; readily distinguished from slow contracture on the one hand, and from the swift, complete tug constituting the familiar all-or-none (maximal) response on the other.

Single fibers of the retrolingual membrane have been stimulated, however, through a single nerve fiber and no trace of submaximal contraction discovered in fatigue or with curare. The muscle fiber thus appears refractory to such grading influence of threshold change as must render uniform nerve impulses capable of grading in a relative sense. All changes attributable to depression of excitability (fatigue, curare) appear to manifest themselves quantally (i.e., in all-or-none steps).

Thus, graded response would appear to be suppressed, for accuracy of integration, by the tendency to complete conduction—a tendency further conserved by the standard maximal relation of the individual nerve impulse to muscle. This marked tendency in higher systems may therefore to great advantage be reconciled with the graded (decremental) properties of less differentiated tissues. "All-or-nothing" may be described as a special case of gradation where the grading range, present but highly attenuated in vertebrate skeletal muscle, apparently attains zero in vertebrate nerve.

*Tetanus in the muscle fiber and the grading influence of stimulation frequency.* FREDERICK H. PRATT.

Two modes of grading appear in photographic records made from small groups of muscle fibers (sartorius of frog) directly stimulated with induction shocks of varying frequency: first, an abrupt accession or elimination of whole fiber activity, where the extent of tetanic contraction of each fiber may be essentially that of the twitch; second, an increment of contraction continuous with frequency, conspicuous in tortoise muscle, and always best shown with gradual changes of frequency (as in the use of an interrupter driven by gravity acceleration and permitted to decelerate through friction).

The recruitment effect in the reflex is thus imitated in the response of the elements of muscle units, and the capacity of the single fiber to summate clearly demonstrated, providing a mechanism for frequency grading by nerve impulses.

*The vocal sac employed as a nerve-muscle preparation.* FREDERICK H. PRATT.

In the male of *Rana pipiens* the vocal sacs (saccular extensions of M. subhyoideus over an evaginated mucous membrane) lie essentially free in the submaxillary lymph sacs and are each readily connected with a cannula in the duct of junction with oral cavity. The supplying branch of the subhyoid nerve (N. omohyoideus, R. subhyoideus) is reached with ease by a small incision posterior to the tympanic membrane, and can be stimulated at will. The vocal sac, freed by opening the lymph sac, survives an extraordinary degree of inflation and then presents a remarkable picture. The muscle fibers, from 10 to 100$\mu$ in diameter, are markedly flattened. The largest, sweeping in wide orbits over the fundus of the sac, are easily transilluminated and are frequently well isolated from neighboring fibers. This preparation lends itself to varied uses. Through a cannula in the duct, solutions and gases may be introduced. Isometric contractions may be studied by leading to a recording capsule, or volume changes by attachment to a volume recorder. Either direct or indirect stimulation can be applied; but above all, the extraordinary width and thinness of these virtually macroscopic skeletal muscle fibers renders intensive study of their responses possible by relatively gross methods.

*The lymph-heart as a facultative effector.* FREDERICK H. PRATT and MARION A. REID.

The innervation of the anterior lymph hearts in *Rana pipiens* is remarkably constant. Section of the supplying branch from the third spinal nerve stops the pulsation of the corresponding organ. Within a few days, however, adequate function is resumed in the absence of the nerve supply. After the lapse of three to four months the nerve is frequently found regenerated. Section again stops the beat. The transition from the myogenic to the neurogenic state is thus experimentally reversible and indicates that the lymph heart is potentially a facultative system with respect to the two modes of activation.

*A transilluminated preparation of the retrolingual membrane in situ.* FREDERICK H. PRATT and MARION A. REID.

A short segment (about $\frac{1}{2}$ cm.) of glass rod is cemented upright to a

slide by means of balsam. The tongue of a decerebrate or anesthetized frog is held horizontally outstretched. An opening is made in its now ventral aspect at a point half way between the margin of the jaw and the tip of the tongue, with care that the overlying anterior area of the retro-lingual membrane is not pierced. The glass upright is thrust upward through the opening so as to permit the membrane to rest on its cross-section without tension. If the preparation is now placed on the stage of the microscope in a Petri dish and covered with Ringer's fluid the membrane may be brilliantly transilluminated. A remarkably diverse set of histological structures is revealed. The preparation lends itself especially to the study of single nerve fibers in relation to muscle units, and admits of micro-stimulation of either structure under active circulation.

A pore electrode, suitably supported, may be substituted for the glass rod and, by proper arrangement of a mechanical stage, be made to bear directly below any structure selected for stimulation and at the same time transmit the requisite light for observation, photography or micromanipulation.

*An investigation of the antagonism of insulin by posterior pituitary extracts as indicated by changes in gastro-intestinal motility.* J. P. QUIGLEY and B. O. BARNES.

Employing unanesthetized trained dogs, fasting 20 to 44 hours, the motility of the stomach and colon has been followed by the balloon method. Using highly purified insulin, confirmation has been obtained of a number of the effects on gastro-intestinal motility previously noted from commercial insulin.

The effect of pituitrin (Parke, Davis & Co.), oxytocin or vasopressin on gastro-intestinal motility in insulinized or non-insulinized dogs appears to be entirely similar. The optimal dose for the gastric effect is 4 to 8 units intravenously of any of the pituitary preparations. When administered to animals with low spontaneous tone, high spontaneous tone or the hyperactivity of the gut produced by insulin, complete inhibition of tone and motility was the invariable result. The inhibition began 15 to 20 seconds after the administration of the pituitrin. In animals under the influence of insulin the blood sugar level remained constant for 3 to 5 minutes after the injection of pituitrin but rose to or above normal within 10 minutes. The blood sugar fell in the period which followed, during which the gut was recovering from the inhibition.

No indication of an augmentation of gastro-intestinal motility was obtained as the result of the administration of pituitary preparations. The mechanism by which pituitrin inhibits the motility of the gut does not appear to be related to its action on carbohydrate metabolism except during the latter part of the inhibition period.

*The Stütz reaction and reflex standing in the spinal cat.* S. W. RANSON and J. C. HINSEY.

In cats the spinal cord was transected at levels varying from L 3 to T 4 and time up to 3 weeks allowed for recovery. The Stütz or support reaction reappeared within the first day, sometimes within a few hours of the transection and within a few days the animal could stand, bearing the weight of the posterior half of the body on the hind legs for periods varying from 20 seconds to 3 minutes. Spinal standing differs from decerebrate

standing in that it is less prolonged and is subject to sudden lapses. The spinal reflex mechanism for standing is complete below the level of the third lumbar segment. The nature of the Stütz reaction, which was discovered by Rademaker in 1926, will be discussed. The relation of spinal standing to decerebrate rigidity and to the normal postural reactions of the intact animal will be considered.

*Spectrophotometric observations on muscle hemoglobin.* G. B. RAY and G. H. PAFF.

The spectrophotometric absorption curves of red muscle extracts have been compared with the reflection curves of fresh living muscle. In both cases the maxima occur at 545 and 580 millimicra, as compared with 540 and 575 for blood hemoglobin. Furthermore the shift of the alpha band when the oxyhemoglobin of muscle is converted to carboxyhemoglobin is 25 A.U. in place of 58 A.U., occurring under similar conditions in hemoglobin from blood. It appears therefore that the two forms of hemoglobin are not identical.

*The effect of Lugol's solution on the metabolic rate of rabbits fed on abnormal human thyroid preparations.* C. G. REZNICHEK.

Studies were made of the effects upon the metabolic rates in rabbits by feeding abnormal human thyroid gland preparations with and without Lugol's solution.

The following preparations were fed in varying daily doses:

1. Fresh hyperthyroid glands desiccated; obtained from the Wisconsin General Hospital, Madison.

2. Preserved (formalin) toxic adenoma and exophthalmic glands desiccated; obtained from and separated into the above grouping by the clinical staff of the Methodist Hospital, Madison.

3. Commercial desiccated thyroid of Lilly (control).

The apparatus used for determining basal metabolism was an open circuit type using room air and determining (by weight) the $CO_2$ and $O_2$. The general laboratory average for normal rabbits was 2.61 calories per kilo per hour. Forty rabbits were used in this study. The normal metabolic rate for each was determined and desiccated gland was then fed in varying daily doses until a high level of metabolism was reached.

All three of the above preparations acted alike in raising the metabolic rate 40 to 100 per cent above normal. Lugol's solution fed simultaneously in doses of 0.06 cc. daily did not protect the animal from an increase in metabolism. Lugol's solution fed alone caused no change in metabolism. Lugol's solution in doses of 0.06 cc. given to animals previously fed on any of the three thyroid preparations did not lower the metabolic rate.

*Studies in the physiology of exercise. III. Further studies in the acid base balance in exercised dogs.* HUGH A. RICE and ARTHUR H STEINHAUS.

Previously we reported a divergence in acid base changes in dogs exercised in swimming and on a treadmill. This we showed to be related to body temperature. When dissipation of body heat was accelerated as in swimming, the blood changes resembled those found in man.

This study was designed to show the effect of variations in the water temperature upon the acid base picture during swimming.

Water temperatures ranging from 15 degrees to 40 degrees Centigrade at 5 degree intervals were used.

Samples of venous blood were taken at frequent intervals before, during, and after the period of swimming, and the serum analyzed for pH by the Hasting's bi-colorimetric method, and for $CO_2$ by the Van Slyke and Neill manometric apparatus.

*The effects of intense x-ray irradiation of the suprarenal gland.* F. T. ROGERS and C. L. MARTIN.

The direct application of x-rays of an intensity estimated as three and one-half human erythema doses to the one exposed adrenal gland of dogs. after the excision of the other gland, produced no subsequent changes in the gland, except a slight fibrosis. No functional disturbances appeared in these animals during a period of observation of from three to twelve months after the irradiation. The application of six to eight doses to the adrenal gland produced no observable functional disorders in dogs kept three months after irradiation, although fibrous proliferation in the gland can be demonstrated microscopically.

Adrenal deficiency symptoms can be induced in dogs by the surgical excision of one gland and giving excessive x-ray doses to the remaining gland, but the lapse of several months may be required for their appearance. These effects are as follows; the gradual onset of a progressive muscular weakness, depression of metabolism, terminal lowering of the blood chlorides and death.

Heavy dosage of x-rays to the exposed adrenal gland induces degenerative changes, first in the medulla of the gland, and then of the zona reticularis and zona glomerulosa and an extensive proliferation of fibrous tissue throughout the gland.

*Relation of rate of rhythmic contractions of rat uterus to oestrus.* SAM ROSENFELD, JR.

Kymographic records of excised rat uterus contracting rhythmically in aerated Tyrode's solution were made by the entire horn, and by the upper and lower half separately.

Seventeen animals were in dioestrus, as determined by vaginal spreads, and twelve in oestrus. The hourly rate in the first group varied between 24 and 69; in the second group, between 11 and 37. The upper half was in all cases the more rapid.

*Vital function levels in normal pregnancy.* A. W. ROWE.

The present report summarizes the results of a study initiated in 1920 as a preliminary or control phase in a comprehensive investigation of the toxemias of pregnancy. During this interval, series of normal pregnant women have been studied throughout the course of the pregnancy and, in an appreciable number of cases, for several months post-partum. Patients have been drawn both from private homes and from institutions. In addition to the complete clinical records the urine, blood chemistry, blood morphology, basal rate, vital capacity measurements, carbon dioxide, sugar tolerance, and a variety of biometric records have been secured. The large number of data prevents presentation in abstract form. Averages will serve as criteria for normal performance in pregnant women.

*Excitable substances in the nerve-muscle complex.* W. A. H. Rushton.

Determinations of the strength-duration curve upon the frog's sartorius after the method of Keith Lucas confirm him in revealing two intersecting curves. These curves have been obtained from the muscle unexcised, soon after excision, and after remaining in the Ringer for 24 hours. One of these curves ($\gamma$) has its time relations (chronaxie) similar to the nerve, the other ($\alpha$) is more than ten times as long. The relative preponderance of the two curves may be altered at will from the exclusion of one through all stages to the exclusion of the other. This may be done by moving the electrodes from the pelvic end of the muscle where only $\alpha$ is obtained by steps to the middle of the neural region when $\gamma$ is most prominent. Or by immersing the muscle in a bath through which the current flows in parallel lines, and varying the angle between muscle fibres and current. When the current flows perpendicularly to the muscle, there is no $\alpha$ curve but $\gamma$ is more easily obtained than at other angles. The $\gamma$ substance therefore appears to be confined to the neural part of the muscle and to lie in a direction perpendicular to the muscle fibres in some cases.

Tetanising the nerve to fatigue block revealed no change of any kind in the $\alpha$ curve for muscle. The $\gamma$ curve disappeared and was replaced by the $\alpha$. The same results were obtained with curare except that the curve suffered a small elevation of rheobase, but there was no significant change of chronaxie even with strong doses acting for many hours. No indication was obtained that $\gamma$ curves ever lengthen gradually into $\alpha$, nor was Lucas' $\beta$ substance ever found.

These observations seem to support the classical conceptions as to the time relations of muscles and nerves and the action of curare, in opposition to the views of Lapicque.

*A quantitative comparison of the growth in chemical composition and structural mass of the human body before birth.* Richard E. Scammon.

There are certain phases of the prenatal chemical growth of the human body that are sufficiently documented with quantitative data to warrant analytic study. The growth of dry substance, ash, and protein, with respect to *body length*, may be adequately represented by forms of the "saturation curve," a variety of the logistic curve developed by Dr. L. J. Reed. The prenatal growth of fat follows an entirely different style of growth and may be better represented by a form of compound interest curve. Expressions of the growth of these components with respect to body length may be easily converted to expressions with regard to time by substitution with appropriate length-age formulae.

When this is done and the absolute and relative rates of increment of the several constituents compared they appear to group in three types; 1, water content, total dry weight and protein weight; 2, weight of ash; and 3, fat weight.

Most of these curves of growth of chemical components are quite different from the growth in weight of the body as a whole, of its regions or parts, and of the various organs. The marked exception to this generalization is the growth in fat content which is almost identical with that of the superficial adipose tissue of the body (as determined by anatomic dissection and weighing).

The distribution of the heights and weights of the specimens used in this study has been compared with that of a group of 2200 observations on

weight and length of the body in prenatal life and has been found to lie well within the limit of error of random sampling.

*A critical experimental study of the phenolic content of adrenal glands and of acid extracts of adrenal tumors.* W. H. SCHULTZ.

An attempt is made to demonstrate the relative value of what are quoted in the literature as qualitative tests for the pressor substance found in adrenal glands and adrenal tumor extracts. Chemical and physical data were presented which support the conception that the author has of the relative specificity of the respective tests discussed.

*On the respiratory quotient of depancreatised ducks.* I. J. SEITZ.

In a previous article[1] we reported our confirmation of the findings of others that the removal of the pancreas from ducks results in an atypical "diabetes mellitus" which is characterized by only a temporary hyperglycemia lasting from 5 to 10 days after which the blood sugar returns to within normal limits of variation. Ducks thus treated gradually lose weight and die in from 6 weeks to 4 months apparently due to the loss of the digestive enzymes contained in the external pancreatic secretion.

In the above article we reported our findings on the insulin content of the tissues of normal and depancreatised ducks. We found that an extract made from the liver, kidneys and muscle of normal ducks according to the Fisher modification[2] of the Doisy, Somogyi, Shaffer[3] method when injected subcutaneously into starved normal rabbits lowered their blood sugar. Extracts from similar tissues of depancreatised ducks did not have such an effect.

In the present study on the respiratory quotient of normal and depancreatised ducks we have employed the Marine modification[4] of the Haldane gravimetric open circuit apparatus. Appropriate tests for airtightness, balance-accuracy, etc., were made and the errors due to variations in temperature were excluded so far as was practicable.

At this writing we have made respiratory quotient studies on seven ducks before and after the removal of the pancreas with the following results:

1. The fasting R.Q. is not markedly influenced by pancreatectomy.
2. The administration of a standard amount of glucose caused a marked rise of the R.Q. in all the depancreatised ducks except one, the rise being only slightly less than in the normal ducks.
3. In this one duck there was a definite reduction in sugar tolerance and no material rise in the R.Q. on administration of glucose.

*The effects of solar irradiation of long visible and ultraviolet wavelengths respectively, with and without supplementary irradiation of various types, on the growth of chicks and the development of parathyroid glands.* CHARLES SHEARD, GEORGE M. HIGGINS and WILLIAM I. FOSTER.

Confinement of chicks behind red-purple corex (Corning Glass G 986 A transmitting 270 to 400 millimicrons) or amber glass (Pittsburgh Amber 48, transmitting 500 millimicrons to end of visible spectrum) does not permit normalcy in development and growth of chicks or of parathyroid

[1] Seitz, I. J. and A. C. Ivy. Proc. Soc. Exper. Biol. and Med., 1929, xxvi, 463.
[2] Fisher, N. F. This Journal, 1923, lvii. 57.
[3] Doisy, E. A., M. Somogyi and P. A. Shaffer. Journ. Biol. Chem., 1923, lv, 31.
[4] Marine, D. Journ. Met. Res., 1922, ii, 29.

glands. Normal parathyroid glands develop only in the presence of both the visible and ultraviolet portions of radiant energy. Supplementary irradiation of chicks, housed behind the amber filter, with an air-cooled quartz mercury lamp (ultraviolet irradiation of approximately 575 ergs each second for each square millimeter) for ten to fifteen minutes a day induces normal growth and development of parathyroid glands. Ultraviolet irradiation of the heads only of chicks is apparently as effective as (and possibly more so than) irradiation of the bodies and legs only. The ultraviolet portion of sunlight, as transmitted by corex, is not sufficient to compensate for the absence of vitamin D in the diet or the ultraviolet content of unfiltered sunlight. Microscopic investigations (Beeke method) on the calcification of tissues (femurs) in chicks about three and five months old respectively, kept behind either amber or corex filters and given various supplementary amounts of irradiation with the quartz mercury arc, show that normal calcification does not occur in chicks receiving only the long wavelengths of sunlight. Further evidence is presented to support our statement that normal growth of chicks and development of parathyroid glands are dependent on both the ultraviolet and visible portions of solar irradiation, all other factors remaining constant so far as is known.

*Reactions of strips from the heart of the monkey* (*Macacus rhesus*). ROBERT W. SIDDLE.

Isolated strips from the hearts of five monkeys have been studied by the method previously reported.[1] The strips were cut from the walls of the right auricle, some through the nodal area, right auricular appendage, right ventricle, right papillary muscle, left auricle, left auricular appendage, left ventricle and left papillary muscle. The monkeys were killed by bleeding under ether anesthesia. From twenty to forty-four minutes, an average of thirty-five, elapsed between the death of the animal and the mounting of the strips in the aerated dextrose Ringer solution. Twenty preparations were made, two of which failed to contract rhythmically. Curiously enough, these were from the auricular appendages. Of the eighteen rhythmic preparations only three gave contractions without some form of additional stimulation. On the addition of small amounts of adrenalin rhythm was established in the quiescent preparations in an average of fifty seconds, the latent periods varying from a few seconds to five minutes. Once established the average duration of the rhythm was 5.5 hours.

In the auricular strips the rates varied from 48 to 132 per minute averaging 80 per minute. In the ventricular strips the rates were comparatively lower averaging 54 per minute. The papillary muscle strips were intermediate between auricle and ventricle, averaging 65 per minute. The two auricular appendages in which rhythm could not be induced gave changes in tone level throughout the experiment and responded to electrical stimulation with a few contractions after each stimulation.

The remarkably constant behavior of the preparations, once the rhythm was established, is well illustrated by a strip of right ventricle which contracted steadily at a rate of 60 per minute for the first nine hours. During this time the amplitude gradually decreased from 15 mm. to 5 mm. After the ninth hour the rate, while relatively constant, increased to a

[1] Greene, C. W. and R. W. Siddle. This Journal, 1927, lxxxi, 460.

somewhat higher level—at 10 hours 84, at 11 hours 108 which was maintained uninterruptedly until the 12th hour. The total time of the rhythm of this strip was 12.5 hours without interruption. The gradient among these strips is in the order auricular wall, papillary muscle, ventricular wall and auricular appendage, a gradient that may be associated with the richness of the supply of nodal tissue.

*The alterations in the gastric function induced by a stimulation from the colon and appendix.* FRED M. SMITH, W. D. PAUL and W. M. FOWLER.

The present study is primarily concerned with the mechanism of the epigastric pain frequently associated with appendicitis and a spastic condition of the colon. In a former investigation, a reflex stimulation of the stomach from the gall bladder, appendix and colon was demonstrated in the dog. The introduction of a few drops of croton oil in either of these structures was followed by an increase in tone and marked increase in the peristaltic activity of the stomach. It was believed that the results of these experiments indicated that the reflex stimulation of the stomach was probably responsible for the type of epigastric pain under consideration in man. The investigation at this point was extended to the patient. Patients with a spastic colon and recurring epigastric distress were first selected for study. A balloon was introduced into the pyloric portion of the stomach and connected with a kymograph. After a control record of the gastric activity was obtained, the colon was distended with air through a rectal tube. There was at once a striking increase in tone and peristalic action of the stomach and the appearance of the typical epigastric distress. The pain corresponded with changes in tone or the passage of a peristalic wave over the pyloric portion of the stomach. This induced alteration in the stomach, and the pain promptly subsided after the deflation of the colon, or was abolished by atropin. In a patient with chronic appendicitis, which was later confirmed by operation, similar changes in the stomach, together with the typical epigastric distress, was induced by massage over the ileocecal region. This patient was fluoroscoped during the palpation of the appendix region and striking changes in the stomach observed and recorded on films. Similar observations were made on patients with irritable colons following the distention of the colon with air. The tender spot in the epigastrium in these patients was confined to the pyloric region and corresponded with the site of the pain. The increase in tone and the peristaltic action of the stomach were frequently accompanied by a prepyloric spasm. Patients with gastric and duodenal ulcer were studied in the same manner. In some, there was no gastric response whatever to distention of the colon. In others, the distention of the colon induced a striking stimulation of the stomach.

*Effect of prolonged treatment with methyl guanidine sulphate on blood pressure of dogs.* LESLIE B. SMITH.

Recent work has indicated that increased blood guanidine might be a possible cause of hypertension. It has been the purpose of this work to test this hypothesis by daily injections of small amounts (0.01 to 0.04 gram per kilo) of methyl guanidine sulphate.

Eight dogs have been used, exclusive of controls. The duration of periods of injection for each dog ranged from a few days to five months. The work on three dogs has been continuous for 16 months. Blood pres-

sure readings were taken 24 hours following injections. All readings were by the auscultatory method, some being taken from subcutaneous carotid loop and others from front leg.

All dogs responded very similarly to the injections. At the beginning of daily injections the systolic pressures rose sharply (15–40 mm. Hg) for few days, then fell (10–20 mm. Hg) for few days. This was followed by a gradual increase for 15 days to 3 months (10–45 mm. Hg) although injections were continued the pressures now very gradually decreased to around normal. When injections were ceased for a period of time the pressure tended to fall toward the normal. When injections were resumed again the dogs responded as before.

This work to date shows that an increased blood guanidine will produce a marked elevated systolic blood pressure. This however is true only for the first few months. After that period of elevated pressure the dogs seem to develop a compensatory mechanism whereby the pressure is gradually returned to normal. This mechanism is lost during a period of no injections because if injections are resumed the pressure changes repeat the same cycle as before.

The pressures of the control dogs remained very constant throughout the period of observation.

*The diuretic effects of various mercurial treatments.* TORALD SOLLMANN and NORA E. SCHREIBER with the collaboration of H. N. COLE, J. A. GAMMEL and J. E. RAUSCHKOLB.

The urine excretion was studied in a series of patients receiving mercurial treatments, in the ordinary clinical courses. The agents may be grouped conveniently as follows, if the terms are understood rather loosely:

*"Organic":* Salyrgan, intramuscular and by vein; Novasurol, intramuscular and by vein; Salicylate, in oil, intramuscular.

*"Ionizable":* Bichloride, oil, muscular (Bichloridol); Binbromide, in NaBr solution, muscular; Oxycyanide, vein.

*"Colloidal":* Metallic (Mercodel); sulphide (Mersulfol).

The patients were not edematous, and were allowed to take water at pleasure.

All these injections resulted in definite diuresis, differing somewhat in degree, but running the same general course; practically regardless of the compound used and whether the injection was into the muscles or vein. If the urine is plotted by the hourly rate of urine flow, it is practically alike for the organic and the ionizable groups, the colloidal group ranking somewhat lower.

Courses of mercurial inunctions also produce a progressive increase in the median urine flow.

The concentration of mercury in the urine is very much greater for the organic than for the ionizable group, although the diuresis is about the same. From this it follows that it may require 1500 times more "organic" than "ionizable" mercury to produce the same diuretic effect; and this in turn suggests that the "organic" group may owe its diuretic action to the liberation of a small quantity of ionizable mercury. The same holds, in principle, for the "colloidal" compounds.

These observations suggest that the mercurial compounds act alike, perhaps by the liberation of ionizable mercury.

*Estimations of the velocity and amount of blood flowing through the shorter paths of the systemic circulation.* ISAAC STARR, JR. and LEON H. COLLINS, JR.

Underlying several methods of estimating the cardiac output of man is the belief that only negligible amounts of blood return to the lungs a second time in 15 seconds. It seemed to us that the evidence offered in support of this belief might be interpreted otherwise; so the question was investigated in animal experiments.

Dogs were anesthetized and kept warm by an electric pad. Carotid blood pressure was recorded. Under artificial respiration the chest was opened, a cannula inserted into the left auricle, a tube run down a carotid into the aorta, and a needle thrust through the wall of the pulmonary artery. At the proper moment a solution of vital red was injected into the left auricle at a constant rate, and simultaneous samples of blood were collected every five seconds from aorta and pulmonary artery.

In the later experiments the apparatus for collecting these samples consisted of two glass tubes, (3 mm. bore; 5 ft. long) placed nearly horizontal, connected at their lower end with lengths of thermometer tubing placed vertically. At the upper end they were attached to a small T tube, the upper limb of which was connected to two burettes through a single stopcock bored with two parallel holes. The other limbs of the T's were connected to the needle and cannula respectively by short lengths of small rubber tubing. The system was filled with mercury. When it was desired to take samples a clamp was removed and the mercury flowing down the vertical tubes drew the columns along the horizontal tubes drawing the blood after. At every 5 seconds or other interval of time the stopcock was opened for an instant and drops of mercury fell into each column of blood, dividing the samples. After the collection the long tubes were broken through the mercury drops, and the samples analyzed for dye content.

From the concentration of dye in the aorta the cardiac output can be calculated (principle of G. N. Stewart); from the relationship between the dye in aorta and pulmonary artery at any time the proportion of blood which had returned to the heart since the beginning of dye injection can be estimated.

The results show that blood rapidly returns to the heart in large amounts under the conditions of the experiment. For example, in an animal whose blood pressure averaged 102 mm. Hg, the cardiac output 160 cc. per kgm. per minute, the amount reaching the pulmonary artery in 10 seconds was 25 per cent of the total cardiac output, in 15 seconds 38 per cent, in 20 seconds 50 per cent, in 25 seconds 53 per cent, in 30 seconds 58 per cent. In five animals whose blood pressure lay between 132 and 100 mm. Hg the amount of blood returning to the heart in 15 seconds averaged 44 per cent. The smallest amount returning was encountered in an animal whose blood pressure varied between 42 and 56 mm. Hg and whose initial cardiac output was 132 cc. per kgm. per minute, viz.: 7 per cent in 10 seconds; 15 per cent in 15 seconds; 24 per cent in 20 seconds; 28 per cent in 25 seconds; 31 per cent in 30 seconds.

When the results of eleven experiments are compared it appears that the amount of blood rapidly returning to the heart varies, as a rule, with the general blood pressure; no relationship with the cardiac output is apparent.

*The effects of extracts of the posterior hypophysis on the water interchange in muscle.* F. R. STEGGERDA.

Measurements were made on the effects of surgical pituitrin, vasopressin and pitocin on the water interchange of the isolated frog's gastrocnemius muscle. In all cases the left gastrocnemius served as the experimental muscle, whereas the right gastrocnemius of the same frog was the control.

After isolation, both the right and left gastrocnemius muscles were placed in normal Ringer's solution for one-half hour. Weighings of the muscles were then made on an analytical balance, after which the muscles were submerged in Ringer's solutions of various strengths. In all cases extracts of posterior pituitary were added to the solution containing the experimental muscle. Successive weighings were made at intervals over a period of more than two hours, whereby percentage variation in muscle weights could be noted and plotted.

Ninety experiments were performed using normal, hypotonic and hypertonic Ringer's solutions containing different amounts of surgical pituitrin, vasopressin and pitocin.

Plotted curves show no appreciable change in weight between the experimental and control muscles. The results tend to show that extracts of the posterior hypophysis have no effect on the permeability of the isolated frog's gastrocnemius to water.

*Adrenalectomy and muscle fatigue.* H. C. STEVENS and J. M. ROGOFF.

Experiments were made upon decerebrate, adrenalectomised cats, in which the fatigue curve of the gastrocnemius muscle was studied. Curves obtained during the period of survival in good health, following double adrenalectomy, did not differ materially from curves obtained with animals that were not subjected to adrenalectomy. The behavior of cats during and following decerebration, the blood pressure and respiratory response, the specific reactions of the gastrocnemius muscle as evidenced by the fatigue curve, the component phases of the twitch myogram and the chronaxie of the tibial nerve and the muscle in the adrenalectomised cats were compared with similar observations, made under the same conditions, in control non-adrenalectomised animals.

*Vasomotor reflexes following parathyroidectomy.* O. O. STOLAND and A. M. LANDS.

The vasomotor reflexes were studied in dogs and cats under ether or amytal anesthesia before and two to ten hours after the removal of thyroids and parathyroids. After thyroparathyroidectomy we found an increase in the irritability of the vasomotor centers as shown by an augmented pressor or depressor response. There was a gradual increase in irritability up to a maximum after which the response was diminished approaching the normal. Clamping the veins from the parts stimulated did not alter the response but denervation completely eliminated response.

Our results indicate that the increased vasomotor responses occurring a few hours following thyroparathyroidectomy is due to increased irritability of the nervous mechanisms involved. We found no evidence in favor of a pressor autocoid substance liberated from the tissues stimulated as suggested by Vincent and Thompson.

*Some comparative changes noted in the specific gravity, hemoglobin, corpuscle count, temperature, pH and $CO_2$ combining power of the blood as result of profuse sweating.* G. A. TALBERT, A. K. SAIKI, C. BORMAN, L. THOMPSON, D. RUDSER and J. BERGMEYER.

The specific gravity of the blood was determined by the Barbour falling drop method in which there was found an increase in blood concentration in 92 per cent of the 169 determinations. In hemoglobin 95 per cent of the 116 deteminations, the red count in 94 per cent of 136 determinations, while the whites 89 per cent of 136 determinations. The pH was increased in 84 per cent of cases while the $CO_2$ combining power of the blood decreased in 81 per cent of the cases. By the Haldane and Newcomer method there was a most uniform increase in lung ventilation with a corresponding blowing off of $CO_2$.

*The sympathomimetic action of guanidine sulphate.* N. B. TAYLOR and S. J. ROSENTHAL.

Guanidine injected intravenously into cats in the dosage of 0.1 gram per kilo was followed by marked hypertonicity of the muscles culminating in convulsive seizures. The muscular hyperexcitability, however, in our opinion only superficially resembled the tetanic convulsions seen after parathyroidectomy. The movements in many instances were of a more purposeful character. Before being completely incapacitated by the muscular spasms the animal would rush to one end of the cage in an attempt to climb the wire lattice. Affective phenomena were sometimes noticeable, e.g., crying, spitting and dilatation of the pupils. In all instances there was evidence of sympathetic stimulation, such as salivation, dilatation of the pupils as just mentioned, erection of the hairs of the back and bushing of the tail, which was frequently lashed from side to side. The pressor effect of guanidine is well known. The possibility occurred to us that the effect upon the skeletal muscles might also be a sympathetic phenomenon and that ergotamin might annul it. Small doses of ergotamin, 0.25 to 0.4 mgm. per kilo, were therefore given at the same time that the guanidine was injected. Convulsions did not appear but the affective reactions were intensified in a remarkable manner. A quiet docile cat was transformed by the dual administration into an enraged, fighting animal. Bristling of the hair, lashing of the tail, spitting, growling, baring of the teeth and extension of the claws was induced by the slightest sensory stimulation. It invariably backed into a corner and faced the observer, and when the cage was turned about orientated itself rapidly to regain the position of defence. The animal appeared perfectly conscious and normal in every way, but the efficiency of its defence reactions was increased in a spectacular fashion.

*Effects of irradiated ergosterol on normal dogs.* E. H. THACKER.

These experiments were undertaken in order to determine whether ergosterol is effective when administered by other routes than the gastro-intestinal tract. Two samples of one commercial preparation and three of another have been used. Subcutaneous administration was attempted on one dog but discontinued because of the development of ulcers. Intravenous injections were constantly effective if doses were large enough. Control animals to which solvent oils were administered were negative in every respect. Dogs were confined on a stock diet. Phosphorus and

calcium determinations were made on arterial blood obtained by cardiac puncture. Inorganic phosphorus fluctuated much as in normal animals but there was no constant effect. When the dosage was increased to about 3 mgm. per kilo, calcium began to fluctuate, the general level rising. After a week to 10 days on a given dosage, there was apparently a certain tolerance established since in some cases there was a fall, in others stasis. With increased dosage there was a further rise, in some cases to 19 or 20 mgm. per 100 cc. of plasma. These high values persist for 10 to 15 days after administration is discontinued. If large doses are administered at once no increase in calcium is obtained, the animals lose weight, become lethargic and may die. If a degree of tolerance is first established, they usually gain weight and become very active but when extreme hypercalcemia occurs, they tend to lose weight, consume less food and become lethargic.

*Action currents in the cerebral cortex of the dog and the rat during reflex activity.* LEE EDWARD TRAVIS and R. YORKE HERREN.

Work which we have been carrying on for the last three or four years indicates that the highest neurological levels are a functional part of the peripheral arcs and that an alteration of the functions of the former alters prejudicially the functions of the latter. These findings are in harmony with those of other workers. In an attempt to make a more direct attack upon this problem we have undertaken the present study.

To date, one dog and seventeen rats have given striking evidence of the appearance of action currents in the cerebral cortex during the elicitation of the patellar and Achilles tendon reflexes. In the dog, for the patellar tendon reflex, action currents appear in the motor cortex 11 sigma after striking the tendon as compared to their appearance in the quadriceps femoris muscle 8.7 sigma after striking the tendon. In the rat, for the Achilles tendon reflex, the action currents appear in all parts of the cerebral cortex 9.5 sigma after the striking of the tendon as compared to their appearance in the gastrocnemius muscle 6.5 sigma after striking the tendon.

These preliminary findings lend further support to the proposition that functionally the highest levels of the central nervous system are a part of the lowest levels.

*The circulatory reaction to gravity in healthy young women. Evidence regarding its precision and its instability.* ABBY H. TURNER, ISABEL NEWTON and FLORENCE HAYNES.

Since recent work regarding the circulatory reaction to gravity is of conflicting outcome, and since a satisfactory explanation of the phenomenon is still lacking, further evidence has been collected looking toward better understanding.

It was first established that the circulatory change on assuming the erect position has very little if any relation to metabolic need. In a series of 31 experiments the basal metabolism was increased by 18 per cent on standing, a change which if interpreted as exercise should raise the heart rate per minute by 2 to 3 beats only. Instead, an average rise of 18 beats was found. In exercise, a widened pulse pressure is expected; instead the pulse pressure here narrowed by an average of 8.6 mm.

If, then, the standing reaction differs fundamentally from the exercise reaction, how precise is it? This second point was tested by tilting 13 well-trained subjects to various angles on a tilting board, with six minutes,

only, in each position. There is for each angle with the horizontal, 15°, 30°, 45°, 60°, 75°, 90°, a particular adjustment, showing a gradual increase in heart rate, a progressive rise in diastolic pressure and an accompanying narrowing of pulse pressure as the vertical is approached.

That this precision for short periods is not indicative of entire control over the circulating blood during prolonged standing is shown by the results of two further series of experiments in which it appeared that the size of the legs increases progressively as quiet standing continues up to an average of 175 cc. during 15 minutes, in 35 experiments, and that a bettered standing reaction may be brought about by using a snug abdominal bandage. The standing heart rate was lessened by 9 beats per minute in 16 bandaged vs. unbandaged experiments, and the pulse pressure increased by 2.5 mm.

*The effect of submersion in water at various temperatures on physiological functions.* W. W. TUTTLE.

Human subjects are submerged in water varying in temperature from 17.5° to 46.5°C. The following physiological functions are studied: 1, respiration; 2, pulse rate; and 3, body temperature.

In the cold water, respiration becomes deep and irregular. As the water temperature is increased the respiration becomes regular and very shallow until the bath reaches body temperature. Higher temperatures cause the respiration to become dyspneic.

Cold water baths cause a sharp decrease in the pulse rate. At approximately body temperature, the rate returns to normal. As the temperature is increased still more, the pulse rate increases, continuing to do so as long as the bath temperature is increased.

The body temperature increases about 1 degree F. in cold baths. As the bath temperature is increased, the body temperature decreases for a time, approaching normal when the bath temperature is equal to body temperature. A further increase in the bath temperature causes an increase in body temperature which continues throughout the experiment.

By varying bath temperatures, the physiological functions studied can be maintained at any desired level.

*The appearance of decerebrate rigidity and other reflexes in young white rats.* JOHN L. ULRICH and HELEN C. COOMBS.

In an extended study of the white rat, detailed observations were made daily upon a number of both normal and decerebrate animals from birth to eighteen days, with regard to the appearance of some of the more important reflexes. The results are given in the table on following page.

The animals were decerebrated so rapidly as to preclude the use of anesthesia. No transient failure of reflexes was observable at this time. The animals were just as lively following decerebration as before it. This falls in line with the observation made by Pike[1] that in young animals, so-called shock is much less than in older ones. Moreover, newborn animals survived decerebration quite as long as the older rats.

Although the extensor tone evoked by the maximal position which elicits the labyrinth reflex is present from birth, it will be noted that decerebrate rigidity is not fully established until about the eleventh day after birth.

[1] Pike, F. H. This Journal, 1909, xxiv, 130.

This confirms Langworthy's statement[1] that decerebrate rigidity begins to develop about the time that animals are first able to support their weight on their legs.

| NUMBER ANIMALS OBSERVED | AGE | AVERAGE WEIGHT | NORMAL | DECEREBRATE |
|---|---|---|---|---|
| | *days* | *grams* | | |
| 7 | 0 | 5 | Movements of progression<br>Labyrinth reflex present | Movements of progression<br>Labyrinth reflex present |
| 4 | 1 | 5 | Labyrinth reflex present | Labyrinth reflex present |
| 3 | 2 | 6 | Labyrinth reflex present | Labyrinth reflex present |
| 3 | 3 | 7 | Labyrinth reflex present | Labyrinth reflex present |
| 3 | 4 | 8 | Labyrinth reflex present | Alternate rhythmic movements of progression |
| 3 | 5 | 8 | Labyrinth reflex present | Same as day before |
| 3 | 6 | 9 | Slight stiffening of fore limbs with dorsal extension of head | Slight opisthotonus |
| 2 | 7 | 9 | Same as day before | Same as day before |
| 4 | 8 | 10 | Same as day before | Neck reflexes first seen |
| 2 | 9 | 10 | Neck reflexes first seen | Neck reflexes better shown |
| 2 | 10 | 11 | Neck reflexes well shown | Opisthotonus |
| 2 | 11 | 12 | Righting reflex on falling first seen | Opisthotonus. Decerebrate rigidity in both fore and hind limbs |
| 2 | 12 | 14 | Teeth present | Decerebrate rigidity well marked; disappears on median longitudinal section of mid-brain |
| 2 | 13 | 16 | Righting reflex on falling sometimes present, sometimes absent | Same as day before |
| 1 | 14 | 18 | Same as day before | Same as day before |
| 1 | 15 | 20 | Eyes open | Alternate rhythmic extension of hind limbs |
| 3 | 16 | 22 | Righting reflex on falling fully developed | Marked opisthotonus<br>Marked decerebrate rigidity |
| 2 | 17 | 23 | Same as day before | Marked decerebrate rigidity |
| 1 | 18 | 24 | Reflexes well established as they appear in the adult | Marked decerebrate rigidity |

*The influence of the pericardium on acute cardiac dilatation produced by vagal stimulation.* E. J. VAN LIERE and GEORGE CRISLER.

Control x-ray pictures of the heart were taken on barbitalized dogs. The vagus was then stimulated so as to obtain cardiac standstill and another x-ray picture was taken. The pericardium was now removed and the animal allowed 2 hours to recover from the effects of the operation. A second control picture was taken which corresponded in size as a rule to the first control picture. The vagus was again stimulated and an exposure was taken when cardiac standstill was obtained. The cardiac silhouettes were traced with a pen and the areas obtained by means of a planimeter.

It was found that the area of the heart was larger during vagal stimulation after the pericardium was removed than it was with the pericardium intact. In a series of 11 dogs the greatest increase in vagal response after

[1] Langworthy, O. P. This Journal, 1924, lxix, 254.

pericardotomy was 9.63 per cent and the least was 1.90 per cent. There were, moreover, no exceptions—the results all pointed in the same direction.

The conclusions drawn were that animals with the pericardium incised show a greater cardiac dilatation when the vagus is stimulated to cause cardiac standstill than do animals with the pericardium intact. And further that the pericardium restrains excessive cardiac dilatation caused by vagal stimulation which is evidence that the pericardium may have a protective action in conditions of extreme stress on the heart.

*A study of the effect of a variety of substances on the rate of sugar utilization.* D. J. VERDA, E. WILLIAMS, G. C. WICKWIRE and W. E. BURGE.

Two goldfish of approximately the same size and with a combined weight of 5 grams were introduced into 100 cc. of 0.1 per cent dextrose solution in beakers. One cc. of urine was added to one beaker; the phosphate precipitate from 1 cc. of urine, after it had been dissolved was added to another; the filtrate of 1 cc. of urine, from which the phosphate had been precipitated was added to another; 100 mgm. of urea to another; 100 mgm. of di-potassium phosphate to another; 100 mgm. of di-sodium phosphate to another; 100 mgm. of sodium chloride to another; 100 mgm. of sodium bicarbonate to another; 3 cc. of 95 per cent ethyl alcohol to another; 100 mgm. pyramidon to another; 100 mgm. amytal to another; 100 mgm. of twilight sleep to another, and chloroform, nitrous oxide, ether and ethylene gases were bubbled through other preparations.

It was found that 1 cc. of urine increased sugar utilization 35 per cent; the phosphate precipitate increased it 36 per cent; the filtrate from the phosphate precipitate had no effect; di-potassium phosphate increased sugar utilization 28 per cent; di-sodium phosphate, 20 per cent; sodium chloride increased it 3 per cent; sodium bicarbonate had no effect; ethyl alcohol increased it 21 per cent; pyramidon increased it 13 per cent; amytal decreased sugar utilization 4 per cent; twilight sleep decreased it 8 per cent; chloroform decreased it 11 per cent; nitrous oxide 1 per cent; ether 10 per cent, and ethylene decreased it 9 per cent.

*The influence of the interval between contractions upon the magnitude of the chemical change in each contraction in striated muscle.* MAURICE B. VISSCHER.

In cardiac muscle we had earlier found and reported that the oxygen consumption per beat is less at rapid rates than at slow. This problem has now been investigated in striated muscle. It is found 1, that stimulated through their nerves, the one at a rapid rate, and the opposite leg muscle at a slow rate, that after the one being stimulated at a fast rate has had exactly the same number of stimuli which completely fatigued the muscle stimulated at the slow rate, it is still able to contract very vigorously. The tension it is still able to develop has been measured with an isometric lever, and compared with the total tension developed by the corresponding muscle. 2. It is found that the lactic acid production per contraction is less at rapid rates than at slow. 3. The oxygen consumption in recovery, measured with the Fenn-Thunberg micro-respirometer, is less after stimulation at short intervals than after a number of more distantly spaced stimulations.

*Metabolic rates and respiratory quotients of rats following the ingestion of dextrin.* LAURENCE G. WESSON.

The metabolic rates and respiratory quotients of 20 rats were obtained for 44 hours following the ingestion of sufficient dextrin to furnish the rats with the required calories for 12 hours. All determinations were made at 28°C. and in the dark after the rat had remained for 3 or 4 days under these conditions. The glycogen stores of the rats were depleted by 18 to 24 hours' fasting before they were fed dextrin. Metabolic rate and respiratory quotient determinations were made both with a Henderson-Haldane gas analysis apparatus, and with an improved form of the closed-circuit apparatus previously described by us. In spite of the more uniform conditions than have previously been used, the metabolic rates and respiratory quotients varied widely in the first hours after feeding the test meal, but, from the 17th hour on, deviations were comparatively small in amount. A tendency toward a diurnal variation in the respiratory quotients was noted. The average, uncorrected respiratory quotient values were 0.73 to 0.74 following the 37th hour, while the metabolic rate values were approximately 720 Cals. per sq. m. body surface per 24 hours.

*Alterations of acid-base values during the ingestion of ammonium nitrate.* MARY WHELAN.

Extensive studies have been made on the alterations in acid-base values in blood and in urine accompanying diuresis produced by calcium chloride and by ammonium chloride. Similar studies have been made on blood and urine before and after the ingestion of ammonium nitrate. The ammonium fraction of the salt is rapidly converted to urea after absorption, leaving a strong acid to be neutralized.

In the urine there is a marked increase in fixed base and chloride, together with excretion of from 80 to 90 per cent of the nitrate ingested. Consequently, during the period of ingestion of nitrate there is a marked increase of total base and total acid over that of the control period and the urine is definitely more acid. During the early part of this period the increasing excretion of fixed base is most marked. It gradually lessens and the ammonia is then definitely increased. The increased excretion of ammonia usually occurs later and is more prolonged than that of the fixed base. Following the period of ingestion of nitrate there is a period of marked retention of both chloride and of fixed base, while the excretion of ammonia continues to be relatively high. In contrast to these changes in the urine, the acid-base equilibrium in the blood plasma remained practically unaltered.

*A comparison of the toxicity and of the effect on sugar metabolism of the alkaloids from several different brands of cigarettes.* G. C. WICKWIRE and W. E. BURGE.

The alkaloids were extracted from 15 grams each of Camels, Lucky Strike, Chesterfield and Old Gold cigarettes. Two goldfish of approximately the same size and with a combined weight of about 5 grams, were introduced into 100 cc. of various dilutions of these alkaloids. It was found that Camels and Chesterfields were twice as toxic as Lucky Strikes and four times as toxic as Old Gold.

The effect of the alkaloids on sugar metabolism was determined in the following way. Two goldfish were introduced into 100 cc. of 0.1 per cent

dextrose solution and sugar determinations were made according to the method of Benedict immediately and after 40 hours, at the end of the experiments. A dilution of 1–5000 of the alkaloids decreased sugar utilization 6 per cent, whereas a dilution of 1–40,000 increased it 25 per cent above the control.

*The quantitative study of the mechanism available to the organism for the dissipation of heat. Preliminary report.* F. H. WILEY and L. H. NEWBURGH.

With the subject under basal conditions, with and without clothing, and under varying conditions of environmental temperature, heat production by indirect calorimetry, skin temperature by means of a thermocouple, and insensible loss of weight by means of a delicate balance were determined. With these data and by means of the formula—insensible loss of weight = water + carbon dioxide − oxygen, the weight of water vaporized was computed and from this value the per cent of the total heat removed by vaporization was calculated.

Results in the nude state: With the environmental temperature between the limits of 20° and 30° (with a constant relative humidity of 20 per cent) the heat production, loss of heat by vaporization, and insensible loss of water were constant. The skin temperature showed a regular increase with the increased environmental temperature. Below 20° the amount of water vaporized decreased and above 30° it rapidly increased.

The heat lost by radiation is governed by physical conditions dependent upon the environmental temperature and skin temperature; whereas heat lost by vaporization is largely under the control of the organisms.

A similar series is being obtained for the clothed subject under basal conditions.

*A critical study of certain factors influencing the specific dynamic action of intravenously administered amino acids and a comparison with oral administration.* C. M. WILHELMJ, J. L. BOLLMAN and F. C. MANN.

In these experiments we have attempted first, to investigate the influence of certain factors which might presumably influence the specific dynamic action of intravenously administered amino acids; second, to compare intravenous and oral administration; and third, to compare the values obtained by us with those obtained by certain other workers. The following conclusions may be drawn.

I. The time required to inject the amino acid solution within time limits ranging from 10 minutes to about 2 hours, causes little or no change in the specific dynamic action provided that the animals are in a stable nutritional condition.

II. Changes in the hydrogen ion concentration of the amino acid solution cause no change in the specific dynamic action since approximately the same values were obtained whether the amino acid was dissolved in water, neutralized with sodium hydroxide to a pH of 7.4, or made distinctly alkaline.

III. Saline solutions which were approximately isotonic with the amino acid solutions caused definite increases in heat production which were equivalent to from 30 per cent to 50 per cent of the specific dynamic action of the amino acid. Physiologic saline solutions are without appreciable influence on the heat production. Glucose solutions, approximately

isotonic with the amino acid solutions cause only slight increases in heat production which is much less than a similar volume of saline solution of approximately the same osmotic pressure. From these results it is obvious that the osmotic pressure effects of the amino acid and glucose solutions are overshadowed by the specific effects.

IV. The same amino acid given to the same dog either intravenously or by mouth causes the same elevation in heat production.

*The chronaxie of the vagus nerve in dogs given methyl guanidine sulphate and in thyroparathyroidectomized dogs.* ROBERT A. WOODBURY.

The rheobase and chronaxie of the vagus nerve to the heart were determined using eighty-two stimuli per second during a stimulation period of two seconds. In dogs treated with small doses (10–50 mgm. per kilo) of methyl guanidine sulphate there followed an increased vagus irritability within ten minutes following intravenous injections and in thirty to sixty minutes following subcutaneous injections. Eighty minutes to three hours after the subcutaneous injections the rheobase and chronaxie readings indicate irritability below normal. Larger doses produce decreased irritability followed by increase to above normal. In thyroparathyroidectomized dogs vagus irritability was increased two to four hours after the removal of the parathyroids and continued high during tremors and mild tetany. Following severe tetany or convulsions the increased rheobase and chronaxie readings indicate a fairly marked fall in irritability.

*The action of adrenaline and certain drugs on the isolated holothurian cloaca.* LELAND C. WYMAN and BRENTON R. LUTZ.

The effects of adrenaline and of certain drugs on the isolated cloaca of Cucumaria frondosa and Stichopus moebii were determined. The effective dose of adrenalin chloride produced a progressive decrease in tone, amplitude and rate, with a final cessation of beat. Adrenaline caused a marked acceleration of the beat of the intestinal blood vessel of Stichopus. Physostigmine, nicotine and histamine produced a progressive increase in tone and a decrease in amplitude and rate. Physostigmine and nicotine sometimes initiated beating in a quiescent preparation. In the case of histamine there was a tendency for a relatively early recovery from its effects. Curare, strychnine and atropine produced a cessation of beat, usually preceded by a decrease in amplitude and rate. The tone was unaffected or slightly decreased by these drugs. Pilocarpine was generally ineffective. Adrenaline produced the typical inhibitory effect after doses of curare or histamine, but was ineffective following physostigmine or nicotine. Following cocaine, ergotoxine, or an ineffective dose of atropine there was evidence of a reversal of the inhibitory effect of adrenaline. Cocaine and ergotoxine produced no visible effect when used alone.

It is pointed out that adrenaline has both inhibitory and excitatory effects on invertebrate tissues. The apparent similarity of the action of the drugs used on the holothurian cloaca to the action of the same drugs on mammalian intestine is discussed. It is concluded, however, in view of the absence of adequate morphological data and the uncertainty of reasoning from the antagonistic action of drugs, that speculation concerning the neuro-muscular organization in holothurians and its physiological relation to that of higher forms is futile.

*The blood volume of the rat in suprarenal insufficiency, anaphylactic shock and histamine shock.* LELAND C. WYMAN and CAROLINE TUM SUDEN.

Blood volume determinations, by the micromethod of Went and Drinker (1929), in twenty-three normal rats averaged 7.6 per cent of body weight, with an average plasma percentage of 56.3. Determinations in blank operated controls, and in suprarenalectomized rats having autoplastic cortical transplants were within the normal range. In thirty-one suprarenalectomized rats, twelve of which were biologically normal due to accessory cortical tissue or having chronic suprarenal insufficiency and nineteen of which died from insufficiency, determinations made when moderate, slight or no symptoms were present showed no significant changes. In eleven rats dying from insufficiency determinations made when severe or terminal symptoms were present showed marked reduction of the plasma, but no significant changes in cell volume.

Blood volume determinations made after a test dose of horse serum, in five sensitized normal rats and eleven sensitized rats having cortical transplants but no medullary tissue, showed a decrease in plasma volume, least in those having no symptoms of anaphylactic shock, greater in those having slight symptoms, and marked in those having severe symptoms. No significant changes in cell volume were observed. Small intraperitoneal doses of histamine were given to seven suprarenalectomized rats having cortical transplants. Six died and one had severe symptoms. Blood volume determinations made after the injections showed marked reduction of plasma percentage and volume. Sixteen normal rats receiving similar or larger doses of histamine had slight or moderate symptoms of shock. Determinations after injections showed reductions of the plasma percentage and volume, the decreases, however, being less than in the suprarenalectomized rats with transplants.

It is suggested that inability to maintain capillary tone and normal permeability, probably due to lack of medullary suprarenal secretion and resulting in reduction of plasma volume, is the chief factor in increased susceptibility of suprarenalectomized rats to anaphylactic and histamine shock.

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# AT THE MENOPAUSE

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## CORPUS LUTEUM OF THE SOW

Lutein Tablets, H. W. & D., are unmodified by treatment with solvents or by exposure to temperatures above animal body heat in the drying process. All separation of extraneous matter is made by mechanical means and all drying is *in vacuo*. The unaltered *corpus luteum* should, therefore, be presented in our products and clinical experience with them should demonstrate their therapeutic activity.

Ovarian dysfunction as evidenced in dysmenorrhea and amenorrhea is also an indication for Lutein medication. Where the diagnosis of such dysfunction was well established, definite therapeutic results have been reported.

WHOLE OVARY TABLETS, H. W. & D.
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are also offered for those who prefer, for certain indications, the use of the whole gland or of the residue remaining after corpus luteum separation.

*Literature furnished on request*

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